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(54) **DIPEPTIDYL KETOAMIDE
META-METHOXYPHENYL DERIVATIVES
AND USES THEREOF**

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None

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2006/0258598 A1 11/2006 Herzner et al.(57) **ABSTRACT**

The present invention relates to dipeptidyl ketoamide
m-methoxyphenyl derivatives and their use in the treatment
of diseases and conditions associated with elevated calpain
activity, such as heart injury caused by infarction, ischemia
with or without reperfusion, neurodegenerative disorders,
malaria, diabetic nephropathy, neurotoxicity induced by
HIV virus, cancer, and fibrotic diseases.

20 Claims, No Drawings

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**DIPEPTIDYL KETOAMIDE
META-METHOXYPHENYL DERIVATIVES
AND USES THEREOF**

CROSS-REFERENCE TO RELATED
APPLICATIONS

This application is filed under the provisions of 35 U.S.C. § 371 and claims the priority of International Patent Application No. PCT/EP2019/061369 filed on 3 May 2019 entitled “DIPEPTIDYL KETOAMIDE META-METHOXYPHENYL DERIVATIVES AND USES THEREOF” in the name of Jesús LLENAS CALVO, et al., which claims priority to European Patent Application No. 18382306.1, filed on 3 May 2018, both of which are hereby incorporated by reference herein in their entirety.

FIELD OF THE INVENTION

The invention relates to dipeptidyl ketoamide m-methoxyphenyl derivatives and their use in the treatment of diseases and conditions associated with elevated calpain activity, such as heart injury caused by infarction, ischemia with or without reperfusion, neurodegenerative disorders, malaria, diabetic nephropathy, neurotoxicity induced by HIV virus, cancer, and fibrotic diseases.

BACKGROUND OF THE INVENTION

Calpains are intracellular proteins belonging to the family of calcium-dependent, non-lysosomal cysteine proteases (proteolytic enzymes) expressed ubiquitously in mammals and many other organisms. Two major forms, calpain 1 and calpain 2, also known as μ -calpain and m-calpain, have been described but further calpain isoenzymes are also postulated (M. E. Saez et al.; *Drug Discovery Today* 2006, 11 (19/20), pp. 917-923). Calpains play an important role in various physiological processes which include the cleavage of different regulatory proteins (K. K. Wang et al., *Trends in Pharmacol. Sci.* 1994, 15, pp. 412-419).

Elevated calpain levels have been measured in various pathophysiological processes, for example: ischemias of the heart, the kidney, the lung, the liver or the central nervous system, inflammations, muscular dystrophies, cataracts of the eyes, diabetes, HIV disorders, injuries to the central nervous system (e.g. brain trauma), Alzheimer’s, Huntington’s, Parkinson’s diseases, multiple sclerosis etc. (see K. K. Wang, above) and infectious diseases such as malaria (IM Medana et al., *Neuropath and Appl. Neurobiol.* 2007, 33, pp. 179-192). It is assumed that there is a connection between these diseases and generally or persistently elevated intracellular calcium levels. This results in calcium-dependent processes becoming hyperactivated and no longer being subject to normal physiological control. A corresponding hyperactivation of calpains can also trigger pathophysiological processes. For this reason, it was postulated that inhibitors of calpain could be of use for treating these diseases.

Yoshida, Ken Ischi et al. (*Jap. Circ. J.* 1995, 59 (1), pp. 40-48), taught that calpain inhibitors had favorable effects following cardiac damage produced by ischemia or reperfusion. Recently, it has been disclosed that calpains activate during myocardial ischemia-reperfusion and contribute to reperfusion injury, as well as the involvement of calpains in post-infarction remodeling and heart failure. After an acute myocardial infarction, the global heart undergoes a series of structural changes, termed post-infarction myocardial remodeling, leading to the incidence of heart failure. Ven-

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tricular remodeling includes the dilatation, hypertrophy, and the formation of a discrete collagen scar. The dysregulation of calpain activity plays an important role in reperfusion injury and myocardial remodeling, suggesting that inhibition of calpain is a potential therapeutic strategy (Ye et al., *PLoS ONE*, 2015, 10(3), e0120178; Kudo-Sakamoto et al., *Journal Biological Chemistry*, 2014, 289(28), pp. 19408-19419). Calpain inhibitors have been reported by Neuhof et al. (*World J Cardiol*, 2014, 6(7), 638-652) as a new prophylactic and therapeutic possibility for patients with myocardial infarction, revascularization and coronary surgery. The study reported by Poncelas et al. (*Cardiovascular Research*, 2017, 113(8), pp. 950-961) confirm this role of calpains and show that sustained pharmacological inhibitor of calpains is a promising therapeutic strategy against adverse post-infarction remodeling

Calpain inhibitors have also been shown to provide a neuroprotective effect in acute neurodegenerative impairments or ischemias such as occur after cerebral stroke (Seung-Chyul Hong et al., *Stroke* 1994, 25 (3), pp. 663-669, and R. T. Bartus et al., *Neurological Res.* 1995, 17, pp. 249-258).

It has also been shown that following experimental brain trauma, calpain inhibitors improve recovery from the memory performance deficits and neuromotor impairments (K. E. Saatman et al., *Proc. Natl. Acad. Sci. USA*, 1996, 93, pp. 3428-3433) and that calpain inhibitors have a protective effect on hypoxia-damaged kidneys (C. L. Edelstein et al., *Proc. Natl. Acad. Sci. USA*, 1995, 92, pp. 7662-6).

More recent studies have shown that calpastatin (the natural inhibitor of calpain) significantly attenuates the pathophysiological effects of activated calpain in a number of diseases such as a) experimental glomerulonephritis (J. Peltier et al., *J A, Soc Nephrol.* 2006, 17, pp. 3415-3423), b) in cardiovascular remodelling in angiotensin II-induced hypertension, c) impaired synaptic transmission in slow-channel congenital myasthenic syndrome (Groshong J S et al., *J Clin Invest.* 2007, 117 (10), pp 2903-2912), d) in excitotoxic DNA fragmentation via mitochondrial pathways (J Takano et al., *J Biol Chem.* 2005, 280 (16) pp. 16175-16184), and in e) necrotic processes in dystrophic muscles (M J Spencer et al., *Hum Mol Gen.* 2002, 11(21), pp 2645-2655).

It is also known that calpains are linked to Alzheimer disease (AD) (Nixon R. A., “The calpains in aging and aging-related diseases”, *Ageing Res Rev.* 2003 October; 2(4):407-18). Calpain 1 is abnormally activated in AD brain (Saito K, et al. “Widespread activation of calcium-activated neutral proteinase (calpain) in the brain in Alzheimer disease: a potential molecular basis for neuronal degeneration”, *Proc Natl Acad Sci USA.* 1993 Apr. 1; 90(7):2628-32). Calpastatin, the endogenous inhibitor of calpains, is significantly decreased in the same neurodegenerative disorder (Nixon R. A., “The calpains in aging and aging-related diseases”, *Ageing Res Rev.* 2003 October; 2(4):407-18). Calpain overactivation triggered by abnormally high calcium levels and calpastatin depletion lead to limited cleavage or degradation of key neuronal proteins in AD (Wang K K, “Calpain and caspase: can you tell the difference?”, *Trends Neurosci.* 2000 January; 23(1):20-6). Calpains indirectly modulate the proteolytic processing of the amyloid precursor protein (APP), a polypeptide thought to play a fundamental role in AD (Siman R. at al. “Proteolytic processing of beta-amyloid precursor by calpain I”, *J Neurosci.* 1990 July; 10(7):2400-11).

Other calpain substrates affected in AD include CaM-kinase II α (CaMK-II α) and PKC, 2 enzymes that regulate

APP phosphorylation and influence its metabolism (Wang K K et al., "Development and therapeutic potential of calpain inhibitors" *Adv Pharmacol.* 1997; 37():117-52); second messenger-related enzymes such as phospholipase C-1, -2, - β 3 (Banno Y. et al, "Endogenous cleavage of phospholipase C-beta 3 by agonist-induced activation of calpain in human platelets", *J Biol Chem.* 1995 Mar. 3; 270(9):4318-24), and cyclin-dependent kinase 5 (Cdk-5) (Lee M S. et al, "Neurotoxicity induces cleavage of p35 to p25 by calpain", *Nature.* 2000 May 18; 405(6784):360-4); transcription factors such as c-Jun, c-Fos, and I κ B (Carillo S, "Differential sensitivity of FOS and JUN family members to calpains", *Oncogene.* 1994 June; 9(6):1679-89 and Lin Y C, "Activation of NF-kappa B requires proteolysis of the inhibitor I kappa B-alpha: signal-induced phosphorylation of I kappa B-alpha alone does not release active NF-kappa B", *Proc Natl Acad Sci USA.* 1995 Jan. 17; 92(2):552-6); and the memory-related gene, cAMP regulatory element-binding protein (CREB) (Mbebi C, "Amyloid precursor protein family-induced neuronal death is mediated by impairment of the neuroprotective calcium/calmodulin protein kinase IV-dependent signalling pathway", *J Biol Chem.* 2002 Jun. 7; 277(23):20979-90). Recently, calpain actions on the GluR1 subunit of AMPA receptors (24), amphiphysin I (25) and suprachiasmatic nucleus circadian oscillatory protein (26), have been shown to modulate synaptic activity and memory formation.

Growing evidence suggests that the cognitive impairment in AD starts long before neuronal death and that signalling between neurons is interrupted at early stages of the disease. The importance of synaptic alterations in AD has been confirmed by studies on transgenic mouse models of AD (Sant'Angelo A, "Usefulness of behavioral and electrophysiological studies in transgenic models of Alzheimer's disease", *Neurochem Res.* 2003 July; 28(7):1009-15) and on amyloid- β peptide-induced (A β -induced) impairment of long-term potentiation (LTP), a widely studied cellular model of learning and memory (Bliss T V. et al, "A synaptic model of memory: long-term potentiation in the hippocampus"; Collingridge G L *Nature.* 1993 Jan. 7; 361(6407):31-9).

Moreover, calpains influence the phosphorylation and proteolysis of tau, another protein associated with AD (Wang K K, "Calpain and caspase: can you tell the difference?", *Trends Neurosci.* 2000 January; 23(1):20-6). Accumulation of phosphorylated tau additionally leads to the formation of so-called neurofibrillary tangles (NFTs) which, together with the well-known amyloid plaques, represent a pathological hallmark of Alzheimer's disease. Similar changes in the tau protein, generally referred to important feature of as tauopathies are also observed in other (neuro) degenerative disorders such as, for example, following stroke, inflammations of the brain, Parkinsonism, in normal pressure hydrocephalus and Creutzfeldt-Jakob disease.

The involvement of calpain in neurodegenerative processes has been demonstrated in transgenic mice with the aid of calpastatin, a specific and natural inhibitor of calpains (Higuchi et al.; *J. Biol. Chem.* 2005, 280 (15), pp. 15229-15237). It was possible with the aid of a calpain inhibitor to reduce markedly the clinical signs of acute autoimmune encephalomyelitis in a mouse model of multiple sclerosis (F. Mokhtarian et al.; *J. Neuroimmunology* 2006, Vol. 180, pp. 135-146). It has further been shown that calpain inhibitors on the one hand block the A β -induced degeneration of neurons (Park et al.; *J. Neurosci.* 2005, 25, pp. 5365-5375),

and in addition reduce the release of the (3-amyloid precursor protein(β APP) (J. Higaki et al., *Neuron*, 1995, 14, pp. 651-659).

With this background, calpain inhibitors represent a novel therapeutic principle for the treatment of neurodegenerative disorders in general and in particular Alzheimer's disease, Parkinson disease, multiple sclerosis, acute autoimmune encephalitis, and Creutzfeldt-Jakob disease.

It has been demonstrated that the HIV-induced neurotoxicity is mediated by calpain (O'Donnell et al.; *J. Neurosci.* 2006, 26 (3), pp. 981-990) and the involvement of calpain in the replication of the HIV virus has also been shown (Teranishi et al.; *Biochem. Biophys. Res. Comm.* 2003, 303 (3), pp. 940-946).

The involvement of calpain in the development of kidney diseases, such as chronic kidney diseases, e.g. diabetic nephropathy, has also been recently demonstrated. Thus, it has been demonstrated by Y. Shi et al. in animal models that the natural calpain inhibitor calpastatin is down regulated during renal ischemia reperfusion (*Am. J. Physiol. Renal Physiol.* 2000, 279, pp. 509-517). Furthermore, A Dnyanmote et al., *Toxicology and Applied Pharmacology* 2006, 215, pp. 146-157, have shown that inhibition of calpain via overexpression of calpastatin reduces the progression of DCVC-induced renal injury in a model of acute renal failure. In addition, Peltier et al. have demonstrated that calpain activation and secretion promotes glomerular injury in experimental glomerulonephritis (*J. Am. Soc. Nephrol.* 2006, 17, pp. 3415-3423). It has also been shown that calpain inhibitors reduce renal dysfunction and injury caused by renal ischemia-reperfusion and thus may be useful in enhancing the tolerance of the kidney against renal injury associated with aortovascular surgery or renal transplantation (P. Chatterjee et al., *Biochem. Pharmacol.* 2005, 7, pp. 1121-1131). On this basis calpain inhibition may be considered a therapy principle useful in the treatment of kidney diseases, such as chronic kidney diseases, e.g. diabetic nephropathy.

Calpain has also been identified as a central mediator essential for parasitic activity. Parasites like *Plasmodium falciparum* and *Toxoplasma gondii* exploit host cell calpains to facilitate escape from the intracellular parasitophorous vacuole and/or host plasma membrane. Inhibition of calpain-1 in hypotonically lysed and resealed erythrocytes prevented the escape of *P. falciparum* parasites, which was restored by adding purified calpain-1. Similarly, efficient egress of *T. gondii* from mammalian fibroblasts was blocked by either small interfering RNA-mediated suppression or genetic deletion of calpain activity and could be restored by genetic complementation (D. Greenbaum et al., *Science* 324, 794 (2009)). Because parasites that fail to escape from their host cells are unable to proliferate, suggesting a strategy for anti-parasitic therapeutics. Pharmacological inhibition of calpain has been shown to exert anti-malarial activity, and hence presents a novel strategy for anti-parasitic strategy such as diseases caused by parasitic infections like malaria or toxoplasmosis (Li et al., *Mol Biochem Parasitol.* 2007; 155(1): 26-32; Jung et al. *Archives of Pharmacal Research* (2009), 32(6), 899-906, Chandramohanadas et al. *Science* (2009), 324, 794).

It has also been reported (Leloup and Wells, *Expert Opin Ther Targets.*, 2011, 15(3), 309-323; Storr et al., *Nat Rev Cancer.*, 2011, 11(5), 364-374; Storr et al., *Pathobiology*, 2015, 82(3-4), 133-141; Selvakumar and Sharma, *Experimental Therapeutic Medicine*, 2010, 1, 413-417; Storr et al., *Oncotarget*, 2016, 30(7), 47927-47937; and Guan et al., *Proc Amer Assoc Cancer Res.*, 2005, 46) that calpains, in par-

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ticular calpain 1 and calpain 2, are involved in a wide variety of common cancers such as breast cancer, colorectal cancer and leukaemia. Calpains 1 and 2 are involved in the development and progression of cancer by allowing cell transformation, the migration and the invasion of tumour cells, and the neovascularization of the tumour. These reports also mention that numerous tumour cells present an abnormally high activity of these calpains. Thus, inhibiting calpain activity would be an efficient way to block the development of a tumour by blocking the transformation and the proliferation of the cells, as well as the vascularization of the tumour.

Fibrosis refers to the accumulation of extracellular matrix molecules that make up scar tissue (collagen-rich extracellular matrix) and is the common feature of fibrotic diseases. Fibrotic diseases can affect any organ, such as kidney, liver, lungs, skin, heart and eye, leading to organ dysfunction and failure and potentially death. In epithelial organs, especially the lung, liver skin and kidney, the replacement of normal functional units of cells with collagen-rich scar tissue and the architectural distortion cause by scar retraction are the major factors in progressive loss of organ function and eventual failure [Friedman et al. *Science Translational Medicine*, 2013, 5, 167]. Calpain activity has been described as essential in wound healing and scar formation [Nassar et al., *PLoS ONE*, 2012, 7(5), e37084]. Calpains have also been reported as being involved in fibrotic diseases [WO 2017/156074 A1; Buckman et al., *Am J Respir Crit Care Med*, 2018, 197, A5747; Potz et al., *J Nat Sci* 2016, 2(9), e218; Ono et al., *Nature Reviews Drug Discovery*, 2016, 15, 854-876], such as liver fibrosis (alcoholic, viral, autoimmune, metabolic and hereditary chronic disease), renal fibrosis (e.g. resulting from chronic inflammation, infections or type II diabetes), lung fibrosis (idiopathic or resulting from environmental insults including toxic particles, sarcoidosis, asbestosis, hypersensitivity pneumonitis, bacterial infections including tuberculosis, among others), interstitial fibrosis, systemic sclerosis (autoimmune disease in which many organs become fibrotic), macular degeneration (fibrotic disease of the eye), pancreatic fibrosis (resulting from, for example, alcohol abuse and chronic inflammatory disease of the pancreas), fibrosis of the spleen (from sickle cell anemia and other blood disorders), cardiac fibrosis (resulting from infection, inflammation and hypertrophy), mediastinal fibrosis, myelofibrosis, endomyocardial fibrosis, retroperitoneal fibrosis, progressive massive fibrosis, nephrogenic systemic fibrosis, fibrotic complications of surgery, especially surgical implants, injection fibrosis and secondary conditions and disease states of fibrosis, such as cirrhosis, diffuse parenchymal lung disease, post-vasectomy pain syndrome and arthrofibrosis, among others. A particular calpain inhibitor, BLD-2660, is currently undergoing phase 1 clinical trials for fibrosis. Thus, inhibiting calpain activity would be an effective strategy for managing fibrotic diseases.

WO 2004/078908 A2 and WO 2005/056519 A1 disclose calpain inhibitors with potential to inhibit calpain 1 in the treatment of different diseases including Alzheimer's disease. In particular, example 17 of WO 2005/056519 A1, which has been assigned the code name SNJ-1945, has proved to be effective in reducing the experimental autoimmune encephalomyelitis (EAE) clinical scores in vivo (Trager N. et al., "Effects of a novel orally administered calpain inhibitor SNJ-1945 on immunomodulation and neurodegeneration in a murine model of multiples sclerosis", *J. Neurochem.* 2014 July; 130(2): 268-279).

There remains a need for new strategies for the management of diseases or conditions associated with elevated

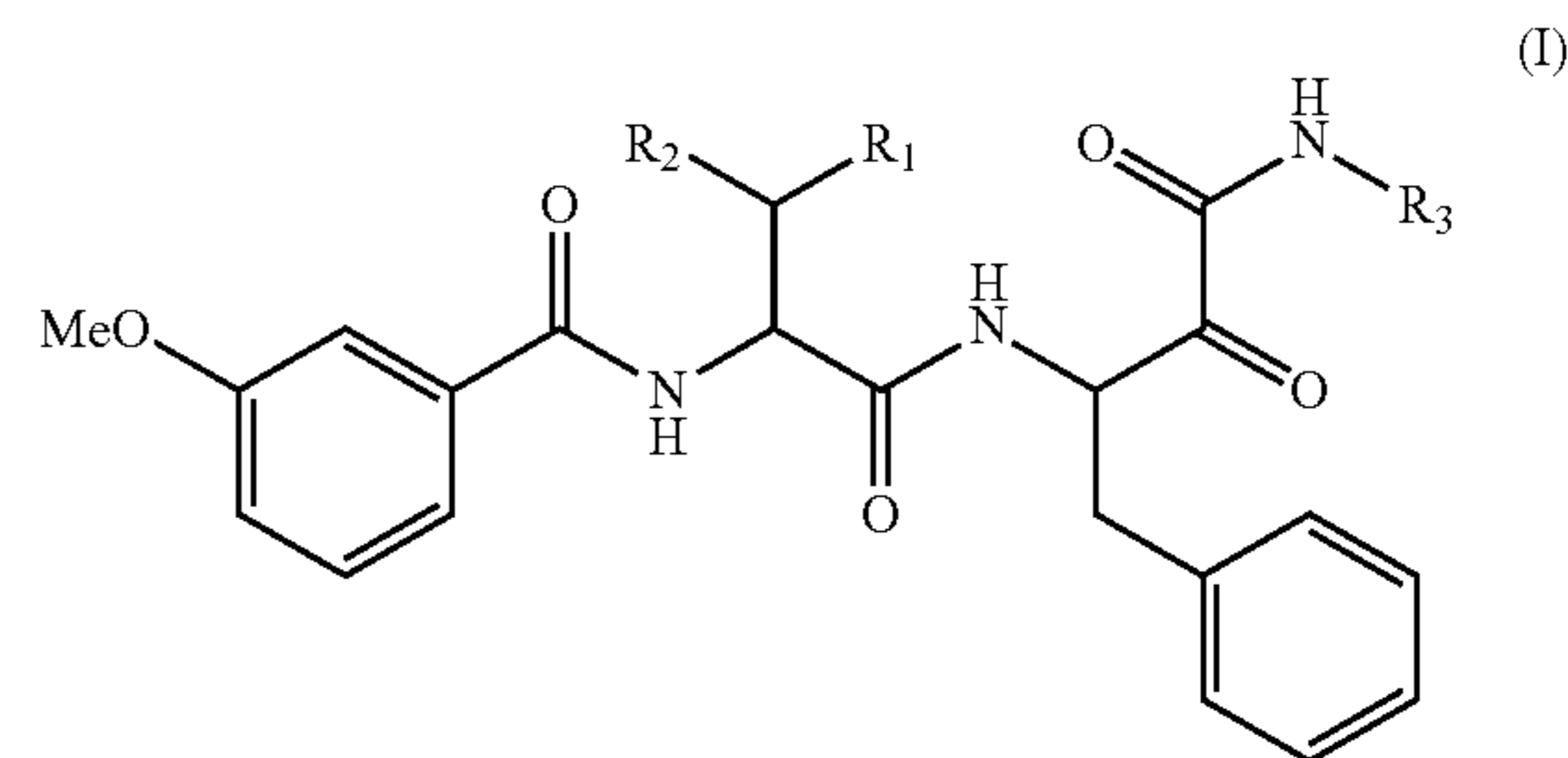
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calpain activity, such as heart injury caused by infarction, ischemia with or without reperfusion; neurodegenerative disorders; malaria; diabetic nephropathy; neurotoxicity induced by HIV virus; cancer; and fibrotic diseases.

SUMMARY OF THE INVENTION

The authors of the present invention have surprisingly found that compounds of formula (I), which have a methoxy group in the meta position of the phenyl ring, are potent calpain-1 inhibitors. The position of this group is of utmost importance for achieving high potency in calpain-1 inhibition, as shown in the comparative examples. Thus, these compounds of formula (I) are useful for the treatment and/or prevention of diseases or conditions associated with an elevated calpain activity, such as heart injury caused by infarction, ischemia with or without reperfusion; neurodegenerative disorders; malaria; diabetic nephropathy; neurotoxicity induced by HIV virus; and cancer.

In a first aspect, the present invention relates to a compound of formula (I):



wherein

R₁ is selected from the group consisting of C₁-C₆ alkyl and C₃-C₆ cycloalkyl,

R₂ is selected from the group consisting of H and C₁-C₆ alkyl,

R₃ is selected from the group consisting of C₁-C₆ alkoxy and C₃-C₆ cycloalkyl,

or a pharmaceutically acceptable salt or stereoisomer thereof, for use in the treatment and/or prevention of a disease or condition selected from the group consisting of heart injury caused by infarction, ischemia with or without reperfusion; neurodegenerative disorders; malaria; diabetic nephropathy; neurotoxicity induced by HIV virus; cancer; and fibrotic diseases.

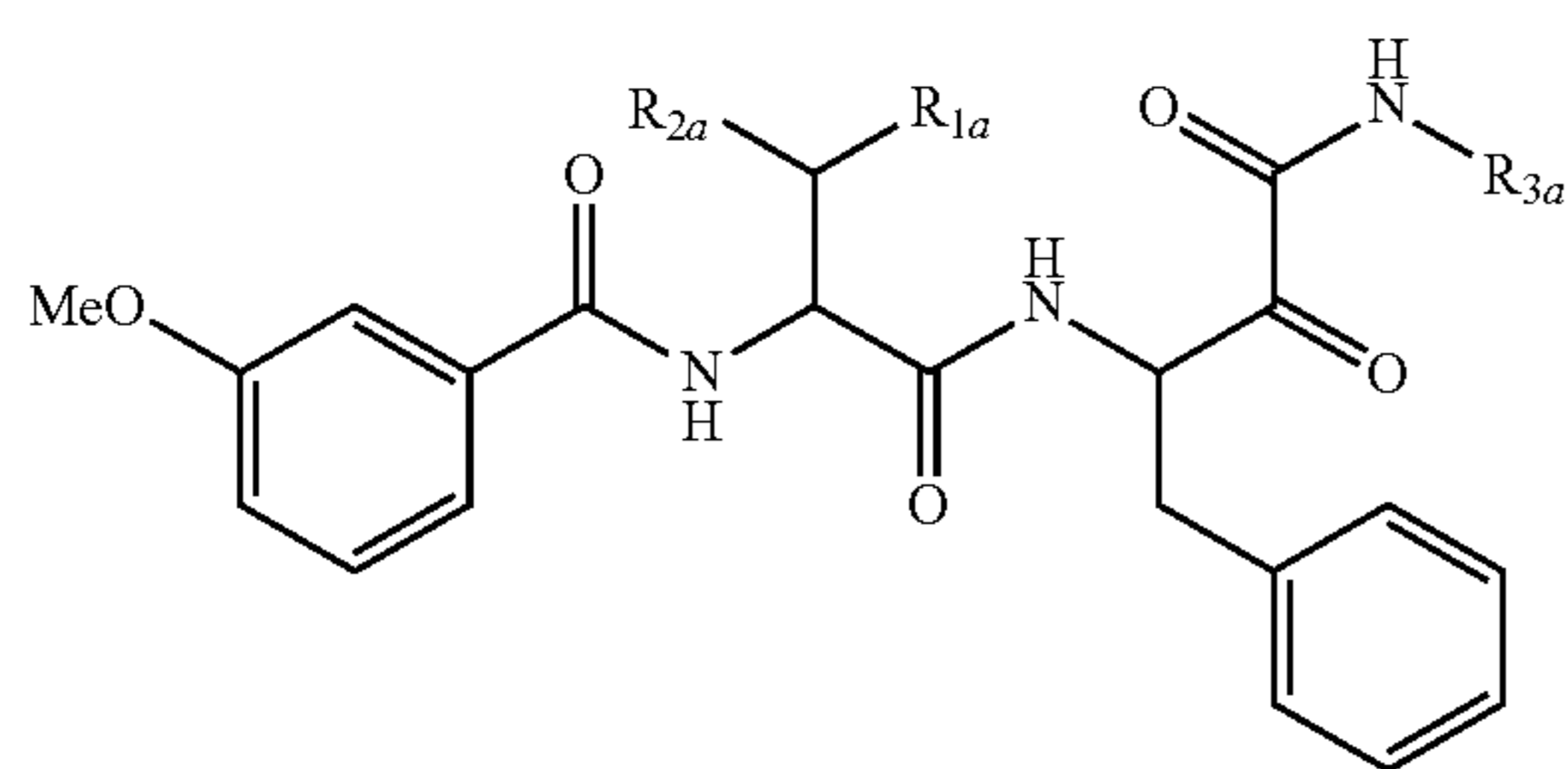
In a second aspect, the present invention relates to a pharmaceutical composition comprising a compound of formula (I) as defined in the first aspect and a pharmaceutically acceptable excipient for use in the treatment and/or prevention of a disease or condition selected from the group consisting of heart injury caused by infarction, ischemia with or without reperfusion; neurodegenerative disorders; malaria; diabetic nephropathy; neurotoxicity induced by HIV virus; cancer; and fibrotic diseases.

In another aspect, the present invention relates to the use of a compound of formula (I) as defined in the first aspect or a pharmaceutical composition comprising said compound and a pharmaceutically acceptable excipient, for the manufacture of a medicament for the treatment and/or prevention of a disease or condition selected from the group consisting of heart injury caused by infarction, ischemia with or without reperfusion; neurodegenerative disorders; malaria; diabetic nephropathy; neurotoxicity induced by HIV virus; cancer; and fibrotic diseases.

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In another aspect, the present invention relates to a method of treatment and/or prevention a disease or condition selected from the group consisting of heart injury caused by infarction, ischemia with or without reperfusion; neurodegenerative disorders; malaria; diabetic nephropathy; neurotoxicity induced by HIV virus; cancer, and fibrotic diseases, the method comprising administering to a subject in need thereof a therapeutically effective amount of a compound of formula (I) as defined in the first aspect or a pharmaceutical composition comprising said compound and a pharmaceutically acceptable excipient.

In a further aspect, the present invention relates to a compound of formula (II):



wherein

R_{1a} is selected from the group consisting of C_1 - C_6 alkyl and C_3 - C_6 cycloalkyl,

R_{2a} is selected from the group consisting of H and C_1 - C_6 alkyl,

R_{3a} is C_3 - C_6 cycloalkyl,

or a pharmaceutically acceptable salt or stereoisomer thereof.

In another aspect, the present invention relates to a pharmaceutical composition comprising a compound of formula (II) as defined in the third aspect and a pharmaceutically acceptable excipient.

In another aspect, the present invention relates to a compound of formula (II) as defined in the third aspect or pharmaceutical composition as defined in the fourth aspect for use in medicine, in particular for the treatment and/or prevention of a disease or condition associated with an elevated calpain activity; preferably selected from the group consisting of heart injury caused by infarction, ischemia with or without reperfusion; neurodegenerative disorders; malaria; diabetic nephropathy; neurotoxicity induced by HIV virus; cancer; and fibrotic diseases.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

Some definitions are included with the aim of facilitating the understanding of the invention.

The term “alkyl” as employed herein alone or as part of another group designates a linear or branched saturated monovalent hydrocarbon chain containing the number of carbon atoms indicated in each case which is typically from of one to six carbon atoms, and preferably from one to three. Examples of alkyls are methyl, ethyl, propyl, 2-propyl, n-butyl, isobutyl, sec-butyl, tert-butyl, n-pentyl, isopentyl, sec-pentyl, tert-pentyl, neopentyl, and the like.

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The term “alkoxy” as employed herein alone or as a part of another group designates an alkyl group as defined above linked through oxygen, i.e. alkyl-O—. Examples of alkoxy include methoxy, ethoxy, isopropoxy, tertbutoxy, and the like.

The term “cycloalkyl” as employed herein alone or as a part of another group designates a monocyclic radical which is saturated or partially saturated, preferably saturated, and which consist solely of carbon and hydrogen atoms and containing the number of carbon atoms indicated in each case which his typically from 3 to 6 and preferably from 3 to 5. Examples of cycloalkyls are cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl.

The term “salt” must be understood as any form of a compound according to the present invention, in which said compound is in ionic form or is charged and coupled to a counter-ion (a cation or anion) or is in solution. This definition also includes quaternary ammonium salts. The definition includes in particular pharmaceutically acceptable salts. The term “pharmaceutically acceptable salt” embraces salts with a pharmaceutically acceptable acid or base, which are synthesized from the parent compound which contains an acidic moiety by addition of a pharmaceutically acceptable base, or which are synthesized from the parent compound which contains a basic moiety by addition of a pharmaceutically acceptable acid. Pharmaceutically acceptable acids include both inorganic acids, for example, hydrochloric, sulfuric, phosphoric, diphosphoric, hydrobromic, hydroiodic, and nitric acid, and organic acids, for example, citric, fumaric, maleic, malic, mandelic, ascorbic, oxalic, succinic, tartaric, benzoic, acetic, methanesulfonic (mesylate), ethanesulfonic, benzenesulfonic (besylate), or p-toluenesulfonic (tosylate) acid. Pharmaceutically acceptable bases include alkali metal (e.g., sodium or potassium) and alkali earth metal (e.g., calcium or magnesium) hydroxides and organic bases, such as alkyl amines, arylalkyl amines, and heterocyclic amines. For instance, pharmaceutically acceptable salts of compounds provided herein are synthesized from the parent compound which contains a basic or an acid moiety by conventional chemical methods. Generally, such salts are, for example, prepared by reacting the free base or free acid forms of these compounds with a stoichiometric amount of the appropriate acid or base, respectively, in water or in an organic solvent or in a mixture of the two.

All stereoisomers of the compounds of this invention are contemplated either alone or as mixtures thereof. The process of preparation can utilize racemates, enantiomers, or diastereomers as starting materials. When diastereomeric or enantiomeric products are prepared, they can be separated by conventional methods, for example, chromatographic or functional crystallization.

Unless otherwise stated, the compounds of the invention are also meant to include compounds which differ only in the presence of one or more isotopically enriched atoms. For example, compounds having the present structures except for the replacement of a hydrogen atom by a deuterium or tritium, or the replacement of a carbon by a ^{13}C - or ^{14}C -enriched carbon or ^{15}N -enriched nitrogen are within the scope of this invention.

Certain compounds of the present invention can exist in unsolvated forms as well as solvated forms, including hydrated forms. In general, the solvated forms are equivalent to unsolvated forms and are encompassed within the scope of the present invention.

Certain compounds of the present invention may exist in multiple crystalline or amorphous forms. In general, all

physical forms are equivalent for the uses contemplated by the present invention and are intended to be within the scope of the present invention.

The term “prevention”, as used herein, refers to the administration of a compound of the invention in an initial or early stage of a disease or condition, or to also prevent its onset.

The term “treatment” is used to designate the administration of a compound of the invention to control disorder progression before or after the clinical signs had appeared. By control of the disorder progression it is meant to designate beneficial or desired clinical results including, but not limited to, reduction of symptoms, reduction of the length of the disorder, stabilization pathological state (specifically avoidance of further deterioration), delay in the disorder’s progression, improvement of the pathological state and remission (both partial and total).

The term “subject”, as used herein, refers to any animal or human that is suffering from one of the diseases or conditions disclosed herein. Preferably, the subject is a mammal. The term “mammal”, as used herein, refers to any mammalian species, including but not being limited to domestic and farm animals (cows, horses, pigs, sheep, goats, dogs, cats or rodents), primates, and humans. Preferably, the mammal is a human being.

The term “heart injury”, as used herein, refers to any damage in cardiac tissue caused by infarction, ischemia with or without reperfusion, such as remodeling.

The term “remodeling”, as used herein, refers to a group of molecular, cellular and interstitial changes that clinically manifest as changes in size, shape and function of the heart resulting from cardiac injury, such as changes in the cavity diameter, mass (hypertrophy and atrophy), geometry (heart wall thickness and shape), areas of scar, fibrosis and inflammatory infiltrate. Cardiac dysfunction is the main consequence of cardiac remodeling, which consists of a pathological substrate for the onset and progression of ventricular dysfunction. This interaction starts with genetic changes in response to a cardiac injury, with reexpression of fetal genes. Consequently, cellular and molecular changes occur, resulting in progressive loss of ventricular function, asymptomatic at first that evolves to signs and symptoms of heart failure. Cardiac remodeling is associated with malignant ventricular arrhythmias, including sustained ventricular tachycardia and ventricular fibrillation. During the first hours after coronary occlusion, disintegration of inter-fibrillar collagen may occur simultaneously with necrosis of myofibrils. The loss of sustaining tissue makes this area more susceptible to distension and deformation. Thinning of the infarcted region and dilation of the cavity occur as a consequence of slippage of necrotic muscle cells and rearrangement of the myocytes across the infarcted wall. This acute ventricular dilation, characterized by thinning and lengthening of the infarct is termed infarct expansion. Infarct expansion increases the likelihood of myocardial rupture and represents an anatomical substrate for aneurysms.

The term “neurodegenerative disorders”, as used herein, refers to disorders which result in progressive degeneration and/or death of neuron cells. Examples of neurodegenerative disorders are Alzheimer’s disease, Parkinson disease, multiple sclerosis, acute autoimmune encephalitis and Creutzfeldt-Jakob disease.

The term “cancer” or “tumour”, as used herein, refers to a broad group of diseases involving unregulated cell growth and which are also referred to as malignant neoplasms. The term is usually applied to a disease characterized by uncontrolled cell division (or by an increase of survival or apop-

tosis resistance) and by the ability of said cells to invade other neighbouring tissues (invasion) and spread to other areas of the body where the cells are not normally located (metastasis) through the lymphatic and blood vessels, circulate through the bloodstream, and then invade normal tissues elsewhere in the body. Depending on whether or not they can spread by invasion and metastasis, tumours are classified as being either benign or malignant: benign tumours are tumours that cannot spread by invasion or metastasis, i.e., they only grow locally; whereas malignant tumours are tumours that are capable of spreading by invasion and metastasis. Biological processes known to be related to cancer include angiogenesis, immune cell infiltration, cell migration and metastasis. Cancers usually share some of the following characteristics: sustaining proliferative signalling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and eventually metastasis. Cancers invade nearby parts of the body and may also spread to more distant parts of the body through the lymphatic system or bloodstream. Cancers are classified by the type of cell that the tumour cells resemble, which is therefore presumed to be the origin of the tumour.

Cancers that can be treated or prevented by the medical uses of the present invention are solid tumours, e.g. colorectal cancer, breast cancer, lung cancer, pancreatic cancer, larynx cancer, tongue cancer, ovarian cancer, prostate cancer, liver cancer, head and neck cancer, oesophageal cancer, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, dysgerminoma, embryonal carcinoma, Wilms’ tumour, cervical cancer, testicular tumour, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, neuroblastoma, retinoblastoma, and leukaemia; preferably breast cancer, colorectal cancer and leukaemia.

The term “leukaemia”, as used herein, refers to a type of cancer of the blood or bone marrow characterized by an abnormal increase of immature white blood cells called blasts and that originates in blood-forming tissue. Leukaemia starts in the bone marrow where developing blood cells, usually developing white cells, undergo a malignant (cancerous) change. This means that they multiply in an uncontrolled way crowding the marrow and interfering with normal blood cell production. Increasing numbers of abnormal cells, called blast cells or leukemic blasts eventually spill out of the bone marrow and travel around the body in the blood stream. In some cases these abnormal cells accumulate in various organs including the lymph nodes, spleen, liver and central nervous system (brain and spinal cord). There are four major kinds of leukaemia: Acute lymphoblastic leukaemia, or ALL; Acute myeloid leukaemia, or AML; Chronic lymphocytic leukaemia, or CLL; Chronic myelogenous leukaemia, or CML.

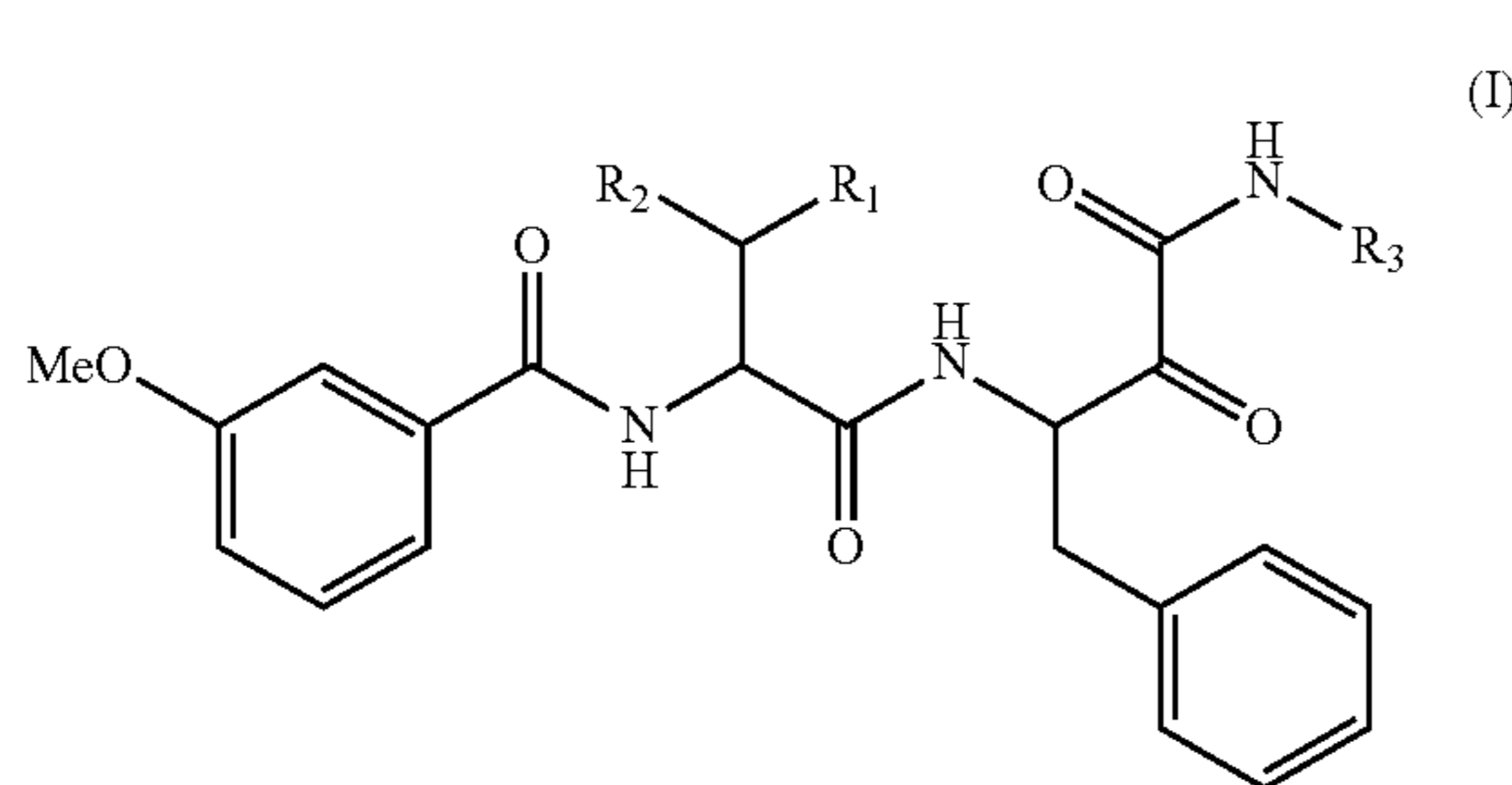
The term “fibrotic disease”, as used herein, refers to a group of diseases which involve the formation of excess fibrous connective tissue in an organ or tissue. Fibrosis results in scarring and thickening of the affected tissue. Fibrosis can occur in many tissues of the body, resulting in a variety of fibrotic diseases, such as cardiac fibrosis, lung fibrosis, liver fibrosis, renal fibrosis, retroperitoneal fibrosis, hypersensitivity pneumonitis, interstitial fibrosis, systemic scleroderma, macular degeneration, pancreatic fibrosis,

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fibrosis of the spleen, mediastinal fibrosis, myelofibrosis, endomyocardial fibrosis, progressive massive fibrosis, nephrogenic systemic fibrosis, fibrotic complications of surgery, chronic allograft vasculopathy and/or chronic rejection in transplanted organs, ischemic-reperfusion injury associated fibrosis, injection fibrosis, cirrhosis, diffuse parenchymal lung disease, post-vasectomy pain syndrome and arthrofibrosis. Preferably the fibrotic disease refers to cardiac fibrosis, lung fibrosis, liver fibrosis, renal fibrosis or retroperitoneal fibrosis.

Compounds of Formula (I)

In the first aspect, the present invention provides a compound of formula (I)



wherein

R_1 is selected from the group consisting of C_1 - C_6 alkyl and C_3 - C_6 cycloalkyl,

R_2 is selected from the group consisting of H and C_1 - C_6 alkyl,

R_3 is selected from the group consisting of C_1 - C_6 alkoxy and C_3 - C_6 cycloalkyl,

or a pharmaceutically acceptable salt or stereoisomer thereof, for use in the treatment and/or prevention of a disease or condition selected from the group consisting of heart injury caused by infarction, ischemia with or without reperfusion; neurodegenerative disorders; malaria; diabetic nephropathy; neurotoxicity induced by HIV virus; cancer; and fibrotic diseases.

In a particular embodiment, R_1 is selected from the group consisting of C_1 - C_3 alkyl and C_3 - C_5 cycloalkyl; preferably from the group consisting of methyl, ethyl, propyl, isopropyl, cyclopropyl, cyclobutyl and cyclopentyl; more preferably from the group consisting of methyl, isopropyl and cyclopropyl; still more preferably from the group consisting of isopropyl and cyclopropyl; the most preferred is cyclopropyl.

In another particular embodiment, R_2 is selected from the group consisting of H and C_1 - C_3 alkyl; preferably from the group consisting of H, methyl, ethyl, propyl and isopropyl; more preferably from the group consisting of H and methyl; the most preferred is H.

In another particular embodiment, R_3 is selected from the group consisting of C_1 - C_3 alkoxy and C_3 - C_5 cycloalkyl; preferably from the group consisting of methoxy, ethoxy, propoxy, isopropoxy, cyclopropyl, cyclobutyl and cyclopentyl; still more preferably from the group consisting of methoxy and cyclopropyl; the most preferred is cyclopropyl. In another particular embodiment, R_3 is C_3 - C_6 cycloalkyl; preferably C_3 - C_5 cycloalkyl; preferably cyclopropyl, cyclobutyl or cyclopentyl; the most preferred is cyclopropyl.

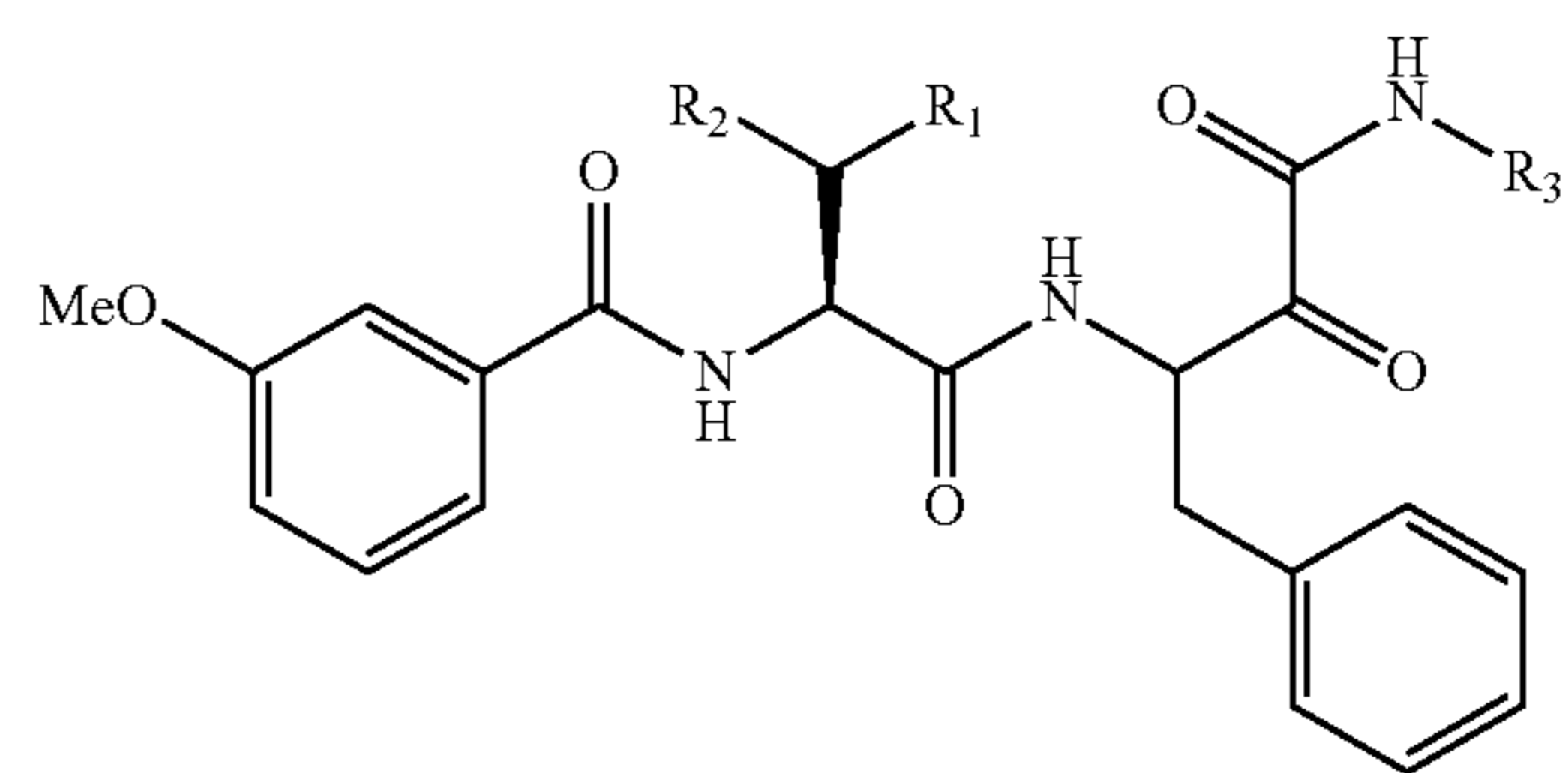
In a particular embodiment, R_1 is selected from the group consisting of C_1 - C_3 alkyl and C_3 - C_5 cycloalkyl; R_2 is selected from the group consisting of H and C_1 - C_3 alkyl; and R_3 is selected from the group consisting of C_1 - C_3 alkoxy and C_3 - C_5 cycloalkyl.

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In another particular embodiment, R_1 is selected from the group consisting of C_1 - C_3 alkyl and C_3 - C_5 cycloalkyl; R_2 is selected from the group consisting of H and C_1 - C_3 alkyl; and R_3 is C_3 - C_5 cycloalkyl.

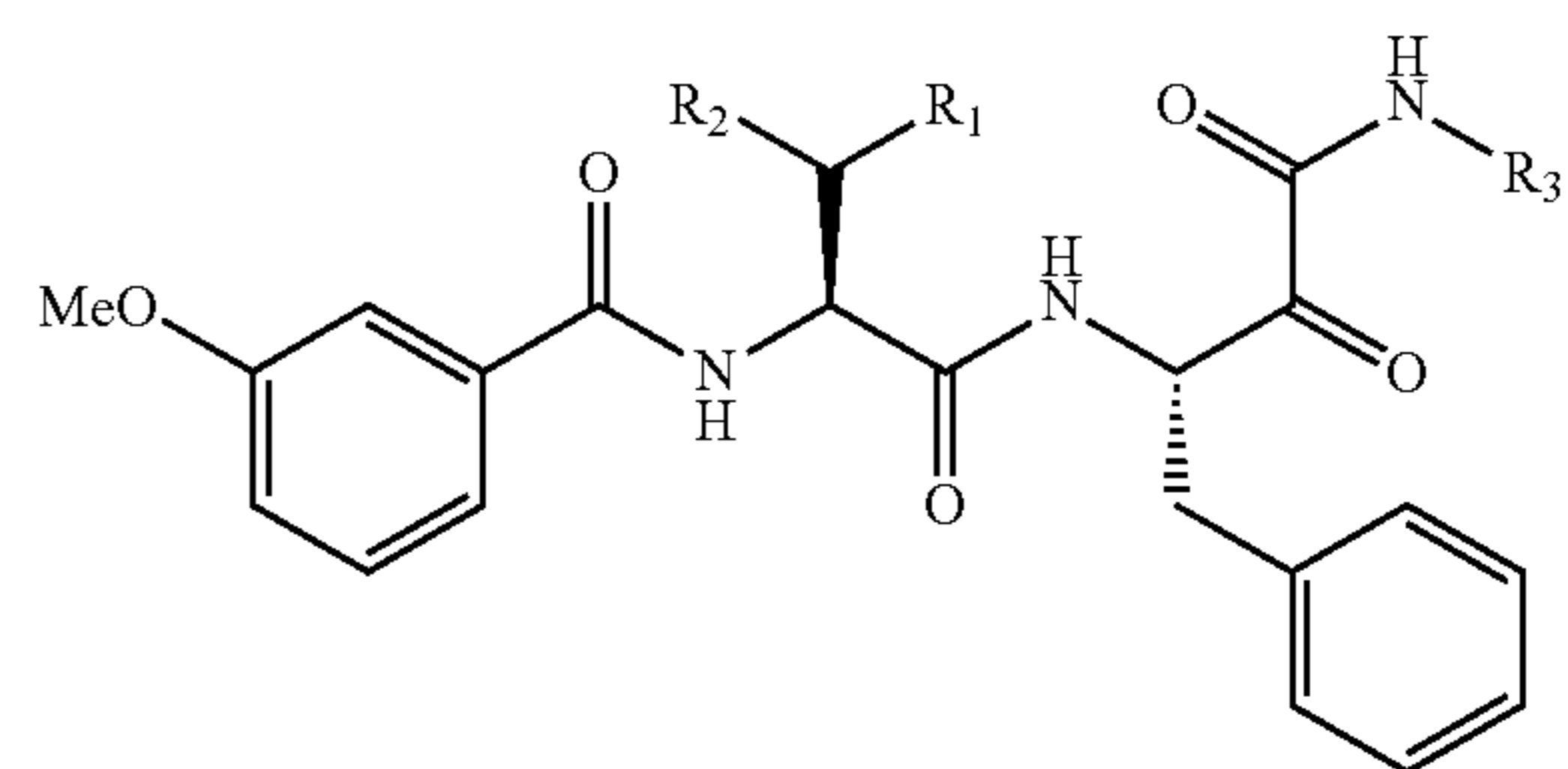
In another particular embodiment, R_1 is selected from the group consisting of isopropyl and cyclopropyl; R_2 is H; and R_3 is cyclopropyl.

In a particular embodiment, the compound of formula (I) is a compound of formula (Ia):



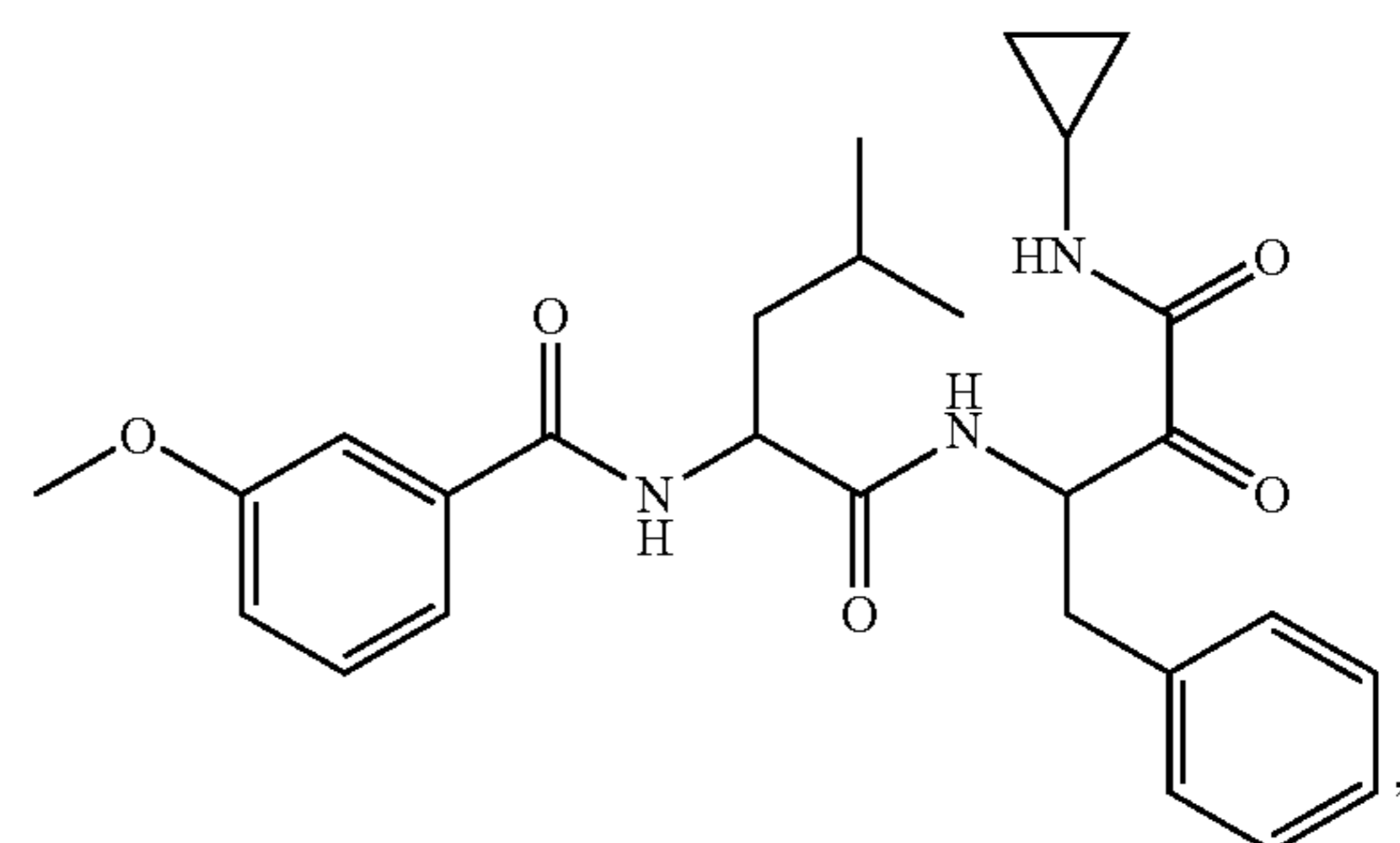
wherein R_1 , R_2 and R_3 have the same meaning as in the compounds of formula (I), or a stereoisomer or a pharmaceutically acceptable salt thereof.

In a particular embodiment, the specific stereoisomer of the compound of formula (I) is a compound of formula (Ib):



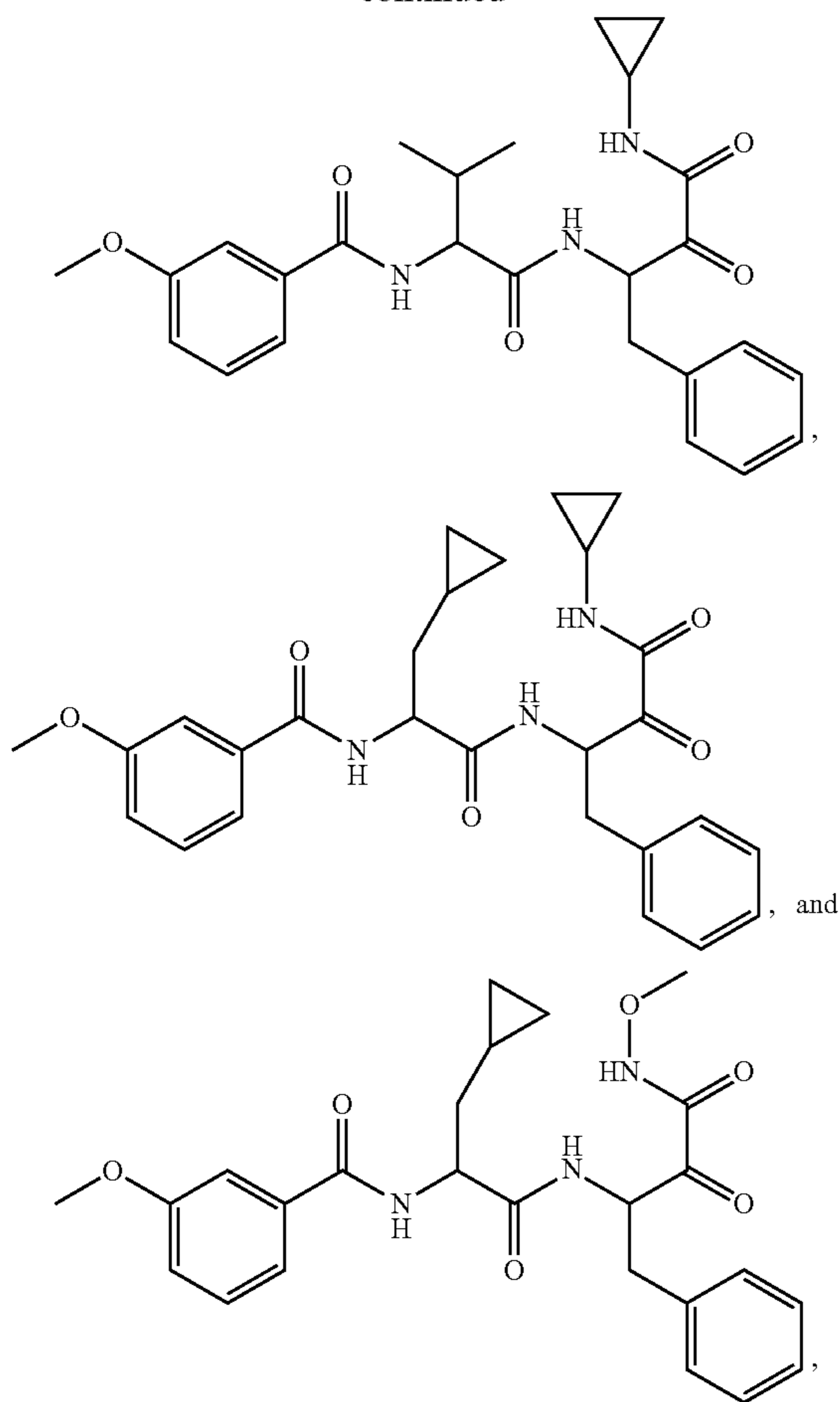
wherein R_1 , R_2 and R_3 have the same meaning as in the compounds of formula (I), or a pharmaceutically acceptable salt thereof.

In a preferred embodiment, the compound of formula (I) is selected from the group consisting of:



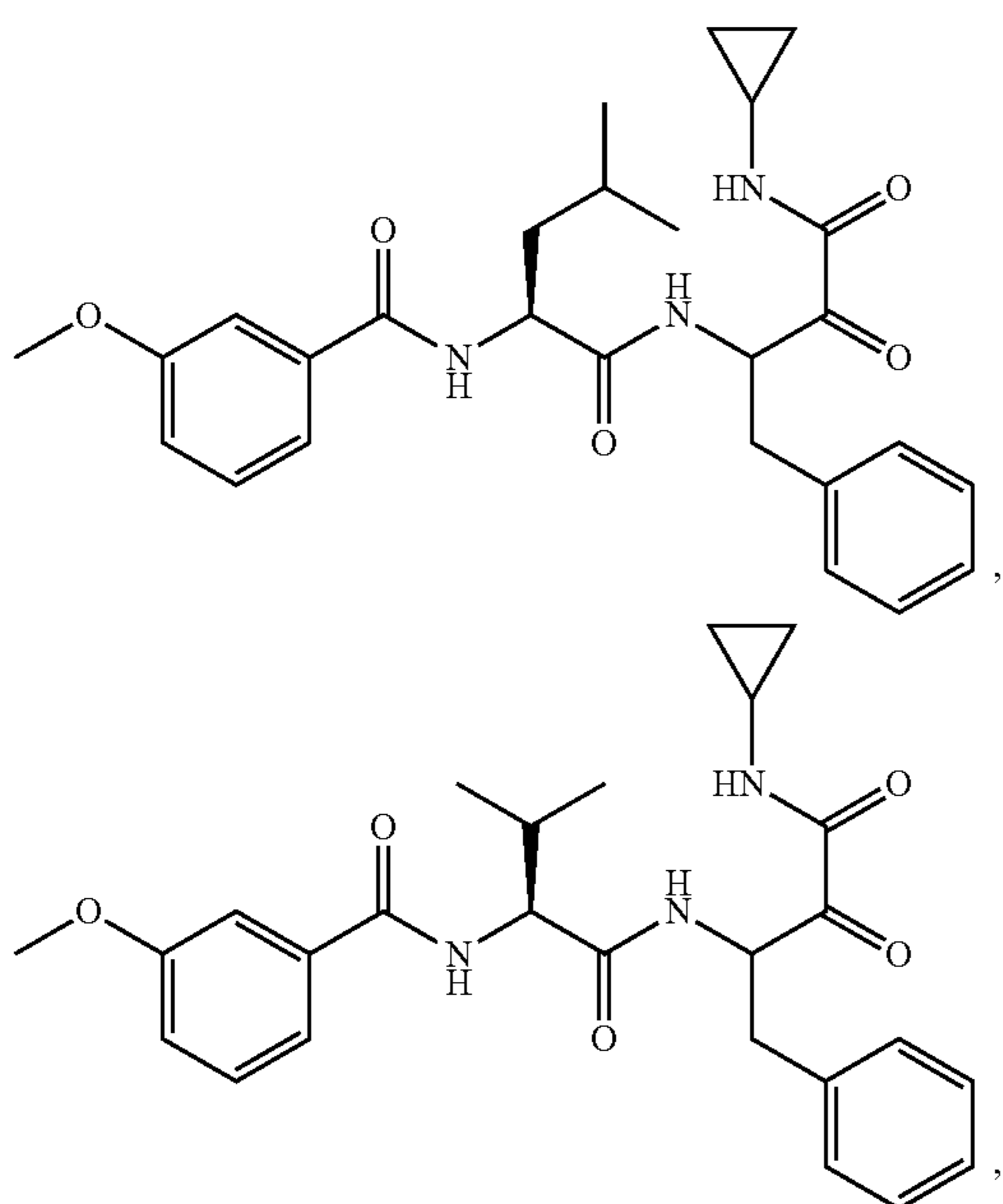
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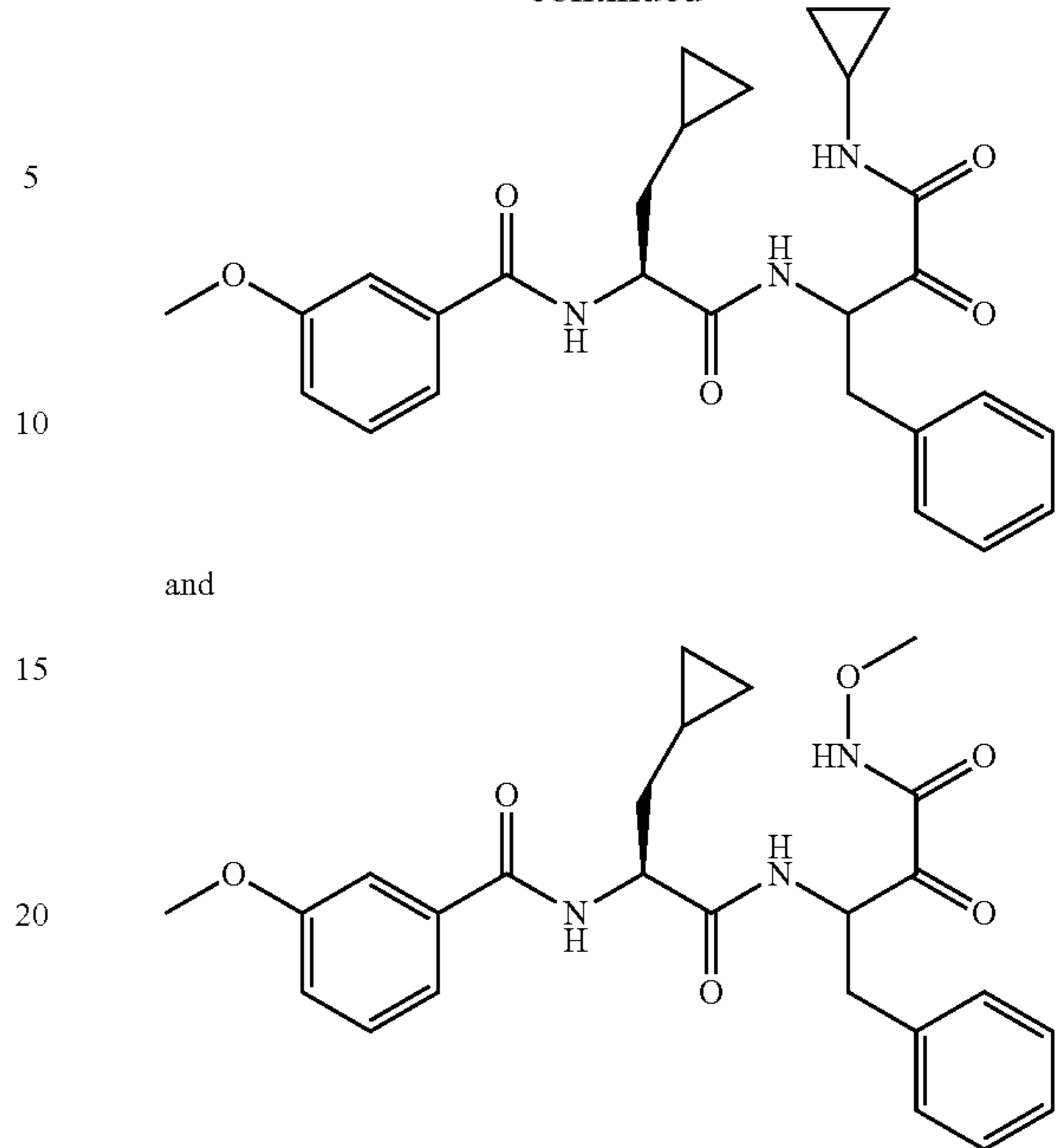
or a stereoisomer or pharmaceutically acceptable salt thereof.

In a particular embodiment, the compound of formula (Ia) is selected from the group consisting of:



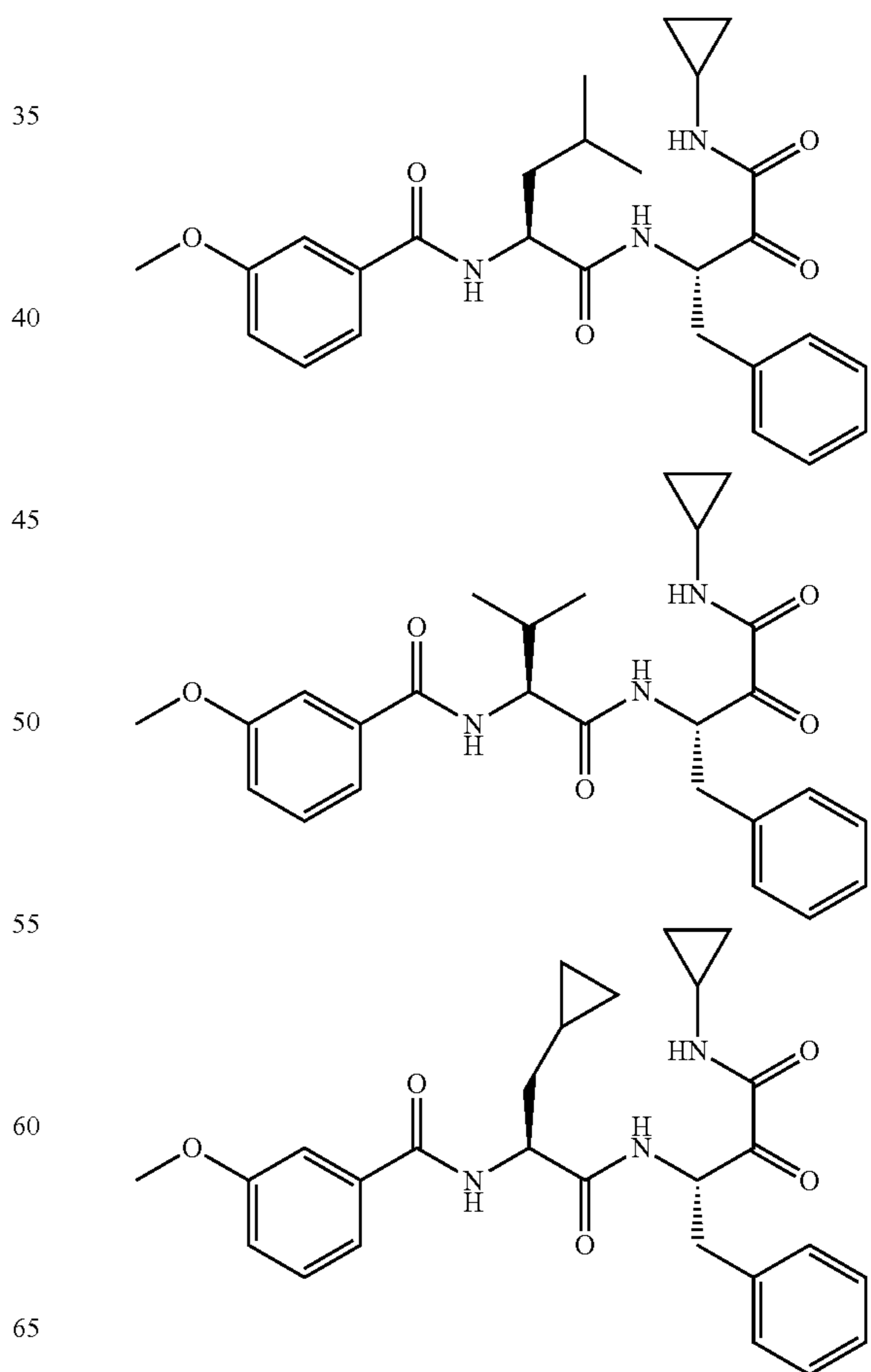
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or a stereoisomer or pharmaceutically acceptable salt thereof.

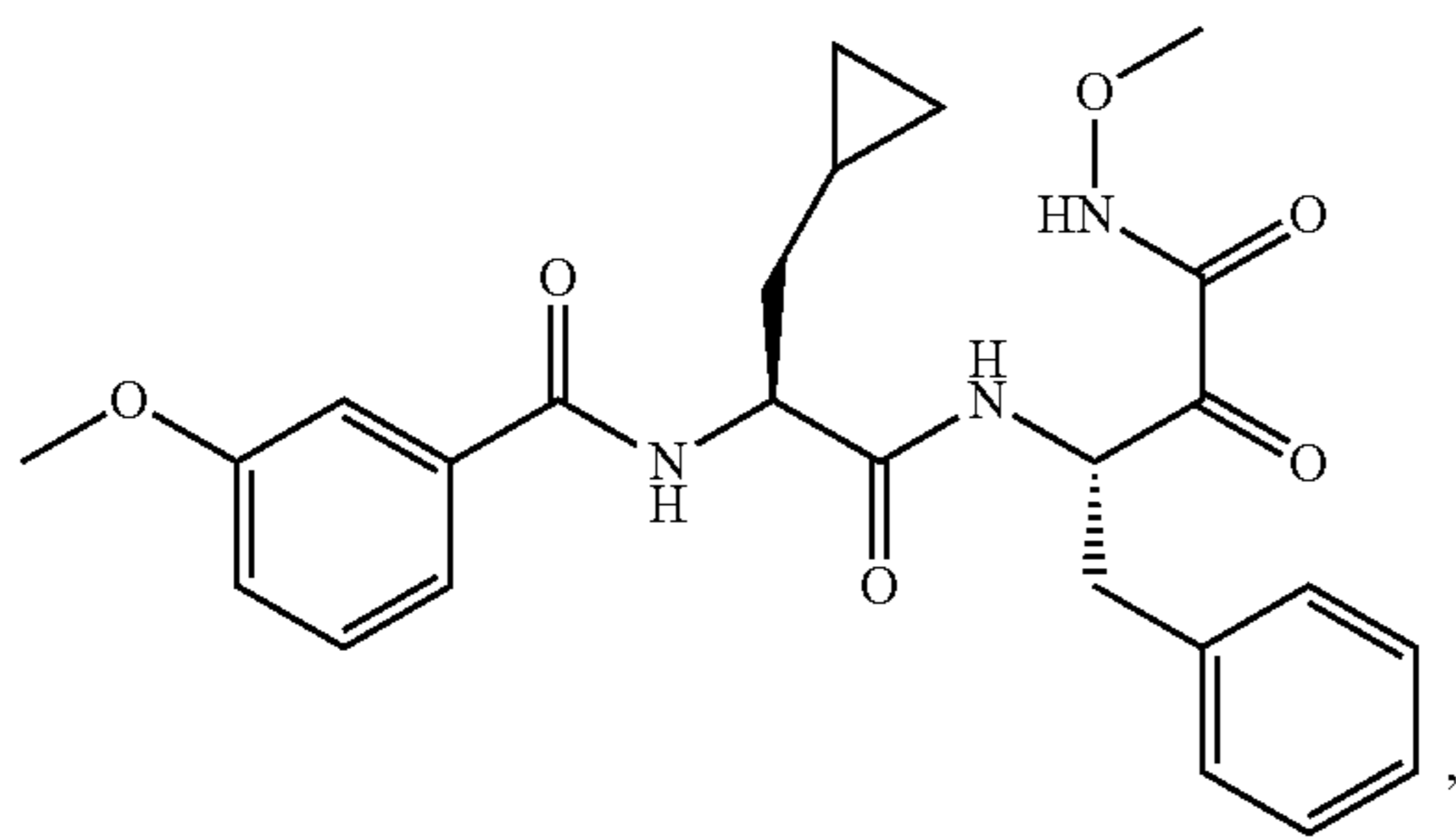
In a particular embodiment, the compound of formula (Ib) is selected from the group consisting of:



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and



or a pharmaceutically acceptable salt thereof.

In a particular embodiment, the compound of formula (I) is for use in the treatment and/or prevention of a disease or condition selected from the group consisting of heart injury caused by infarction, ischemia with or without reperfusion; neurodegenerative disorders; malaria; diabetic nephropathy; neurotoxicity induced by HIV virus; and cancer.

In a preferred embodiment, the compound of formula (I) is for use in the treatment and/or prevention of heart injury caused by infarction, ischemia with or without reperfusion, preferably remodeling after myocardial infarction.

In a particular embodiment, the compound of formula (I) is for use in the treatment and/or prevention of a neurodegenerative disorder selected from the group consisting of Alzheimer's disease, Parkinson disease, multiple sclerosis, acute autoimmune encephalitis, and Creutzfeldt-Jakob disease.

In another particular embodiment, the compound of formula (I) is for use in the treatment and/or prevention of a cancer selected from the group consisting of breast cancer, colorectal cancer and leukaemia.

In another particular embodiment, the compound of formula (I) is for use in the treatment and/or prevention of a fibrotic disease selected from the group consisting of cardiac fibrosis, lung fibrosis, liver fibrosis, renal fibrosis and retroperitoneal fibrosis.

In a second aspect, the present invention relates to a pharmaceutical composition comprising a compound of formula (I) as defined in the first aspect and a pharmaceutically acceptable excipient for use in the treatment and/or prevention of a disease or condition selected from the group consisting of heart injury caused by infarction, ischemia with or without reperfusion; neurodegenerative disorders; malaria; diabetic nephropathy; neurotoxicity induced by HIV virus; cancer; and fibrotic diseases.

In another aspect, the invention relates to the use of a compound of formula (I) as defined above, or pharmaceutically acceptable salt or stereoisomer thereof, for the manufacture of a medicament for the treatment and/or prevention of a disease or condition selected from the group consisting of heart injury caused by infarction, ischemia with or without reperfusion; neurodegenerative disorders; malaria; diabetic nephropathy; neurotoxicity induced by HIV virus; cancer; and fibrotic diseases.

In a further aspect, the present invention relates to a method of treatment and/or prevention a disease or condition selected from the group consisting of heart injury caused by infarction, ischemia with or without reperfusion; neurodegenerative disorders; malaria; diabetic nephropathy; neurotoxicity induced by HIV virus; cancer; and fibrotic diseases, which comprises administering to a subject in need thereof

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a therapeutically effective amount of a compound of formula (I) as defined above or a pharmaceutically acceptable salt or stereoisomer thereof.

In particular embodiments of the uses and methods of treatment defined above, the disease or condition is selected from the group consisting of heart injury caused by infarction, ischemia with or without reperfusion; neurodegenerative disorders; malaria; diabetic nephropathy; neurotoxicity induced by HIV virus; and cancer.

In other particular embodiments of the uses and methods of treatment defined above a preferred embodiment, the disease or condition is heart injury caused by infarction, ischemia with or without reperfusion, preferably remodeling after myocardial infarction.

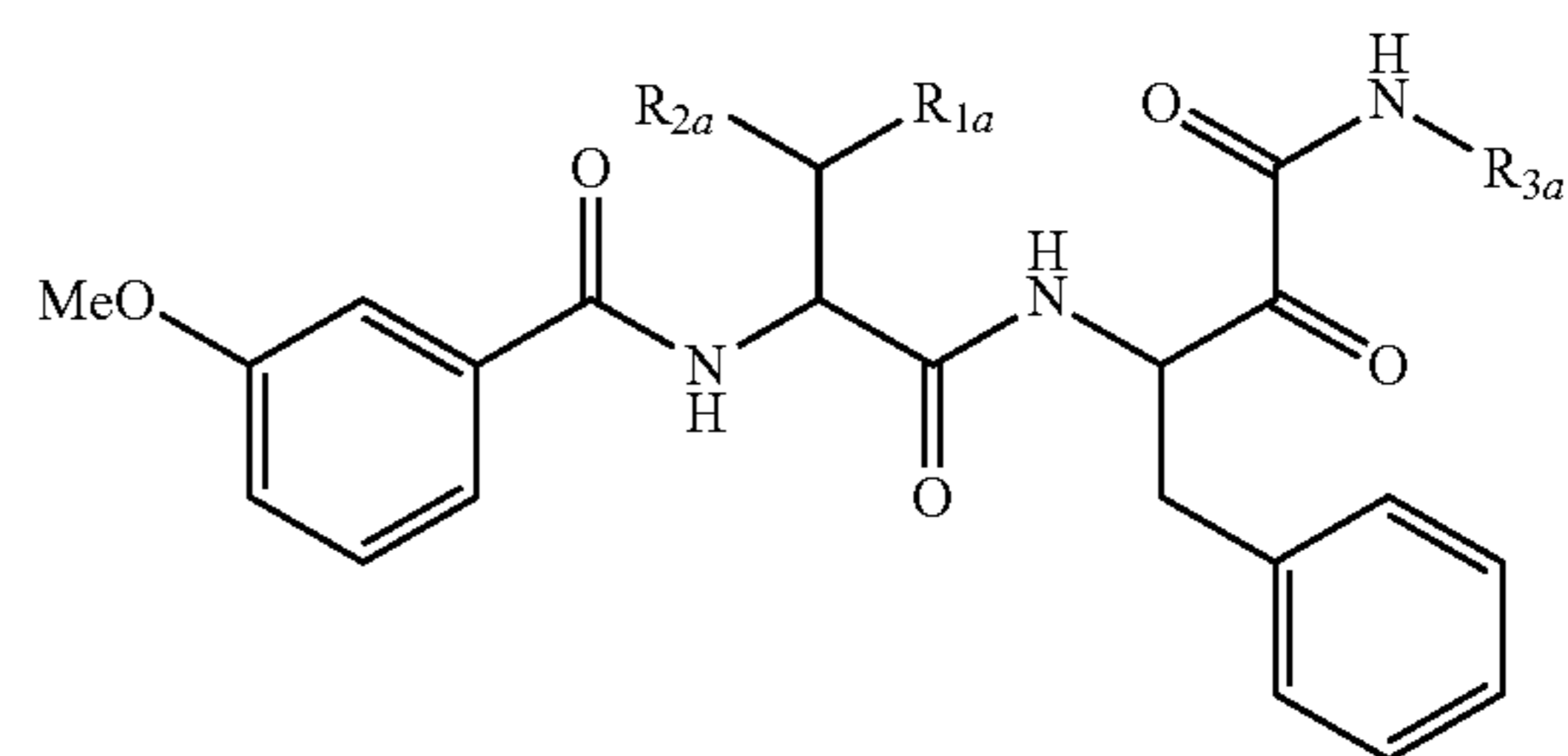
In other particular embodiments of the uses and methods of treatment defined above the neurodegenerative disorder is selected from the group consisting of Alzheimer's disease, Parkinson disease, multiple sclerosis, acute autoimmune encephalitis, and Creutzfeldt-Jakob disease.

In other particular embodiments of the uses and methods of treatment defined above the cancer is selected from the group consisting of breast cancer, colorectal cancer and leukaemia.

In other particular embodiments of the uses and methods of treatment defined above the fibrotic disease is selected from the group consisting of cardiac fibrosis, lung fibrosis, liver fibrosis, renal fibrosis and retroperitoneal fibrosis.

Compounds of Formula (II)

In the third aspect, the present invention provides a compound of formula (II):



wherein

R_{1a} is selected from the group consisting of C_1 - C_6 alkyl and C_3 - C_6 cycloalkyl,

R_{2a} is selected from the group consisting of H and C_1 - C_6 alkyl,

R_{3a} is C_3 - C_6 cycloalkyl,

or a pharmaceutically acceptable salt or stereoisomer thereof.

Compounds of formula (II) are a subgroup of compounds of formula (I).

In a particular embodiment, R_{1a} is selected from the group consisting of C_1 - C_3 alkyl and C_3 - C_5 cycloalkyl; preferably from the group consisting of methyl, ethyl, propyl, isopropyl, cyclopropyl, cyclobutyl and cyclopentyl; more preferably from the group consisting of methyl, isopropyl and cyclopropyl; still more preferably from the group consisting of isopropyl and cyclopropyl; the most preferred is cyclopropyl.

In another particular embodiment, R_{2a} is selected from the group consisting of H and C_1 - C_3 alkyl; preferably from the group consisting of H, methyl, ethyl, propyl and isopropyl; more preferably from the group consisting of H and methyl; the most preferred is H.

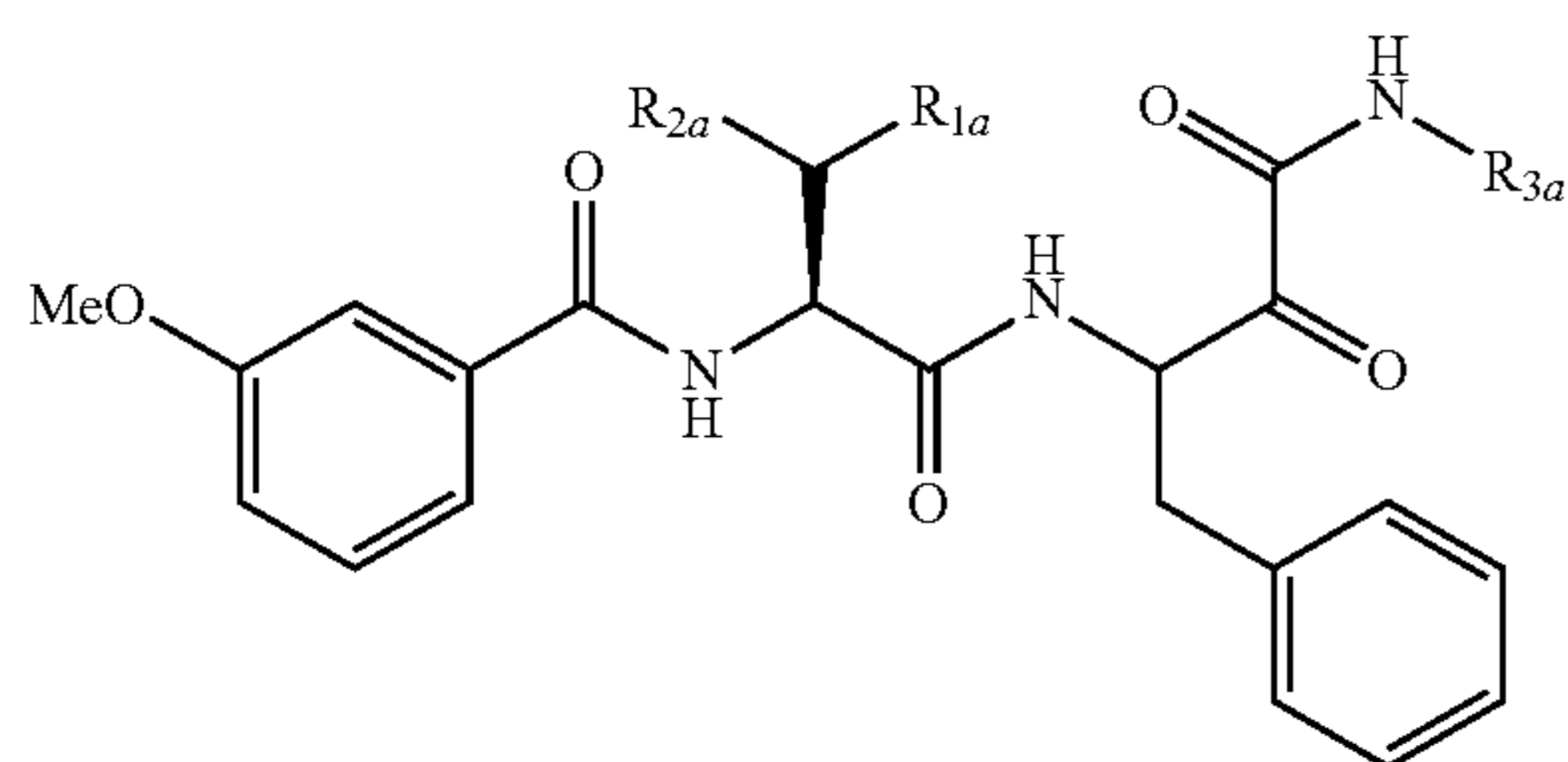
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In another particular embodiment, R_{3a} is C_3 - C_6 cycloalkyl; preferably C_3 - C_5 cycloalkyl; preferably cyclopropyl, cyclobutyl or cyclopentyl; the most preferred is cyclopropyl.

In a particular embodiment, R_{1a} is selected from the group consisting of C_1 - C_3 alkyl and C_3 - C_5 cycloalkyl; R_{2a} is selected from the group consisting of H and C_1 - C_3 alkyl; and R_{3a} is C_3 - C_5 cycloalkyl.

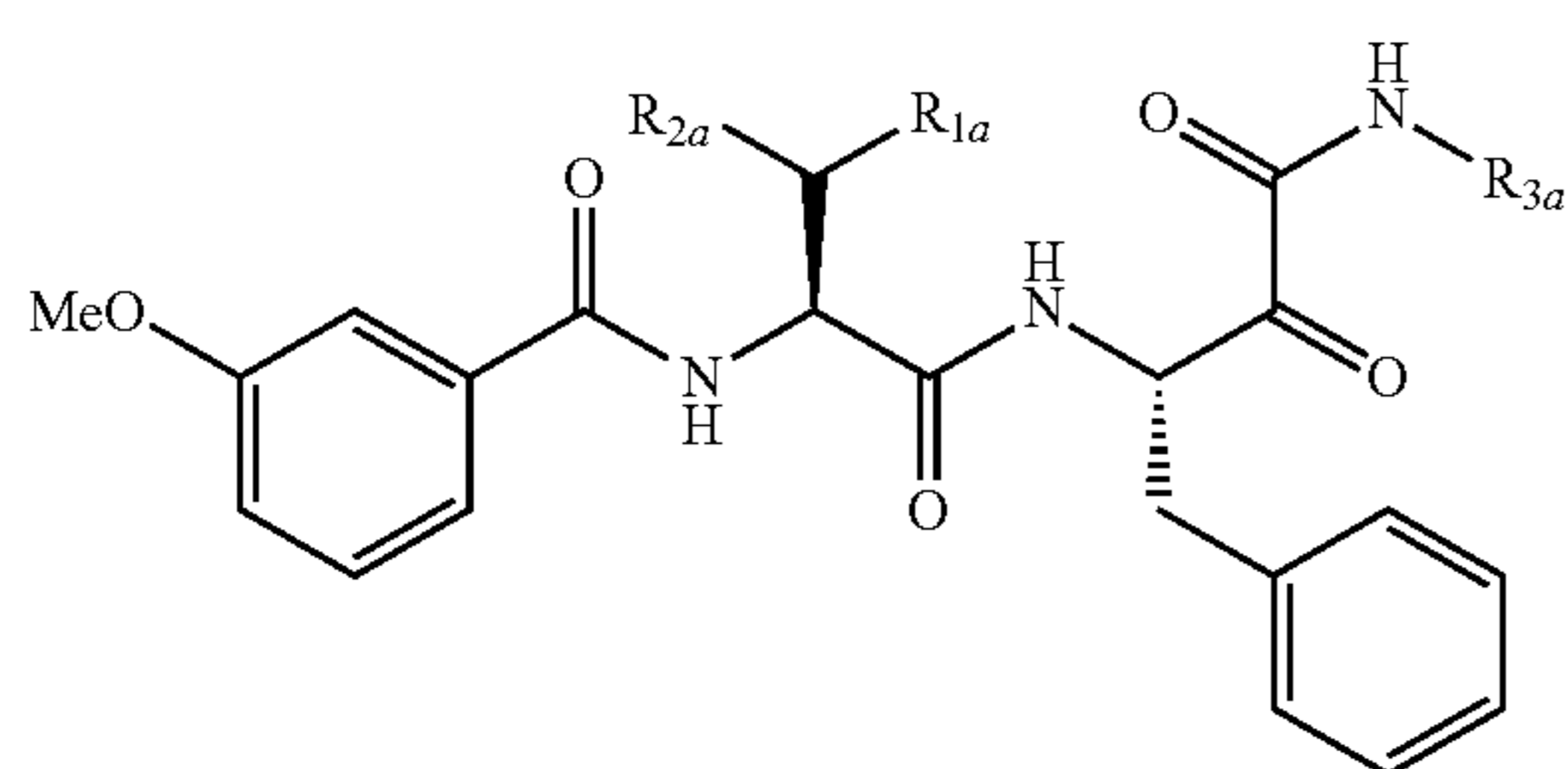
In another particular embodiment, R_{1a} is selected from the group consisting of isopropyl and cyclopropyl; R_{2a} is H; and R_{3a} is cyclopropyl.

In a particular embodiment, the compound of formula (II) is a compound of formula (IIa):



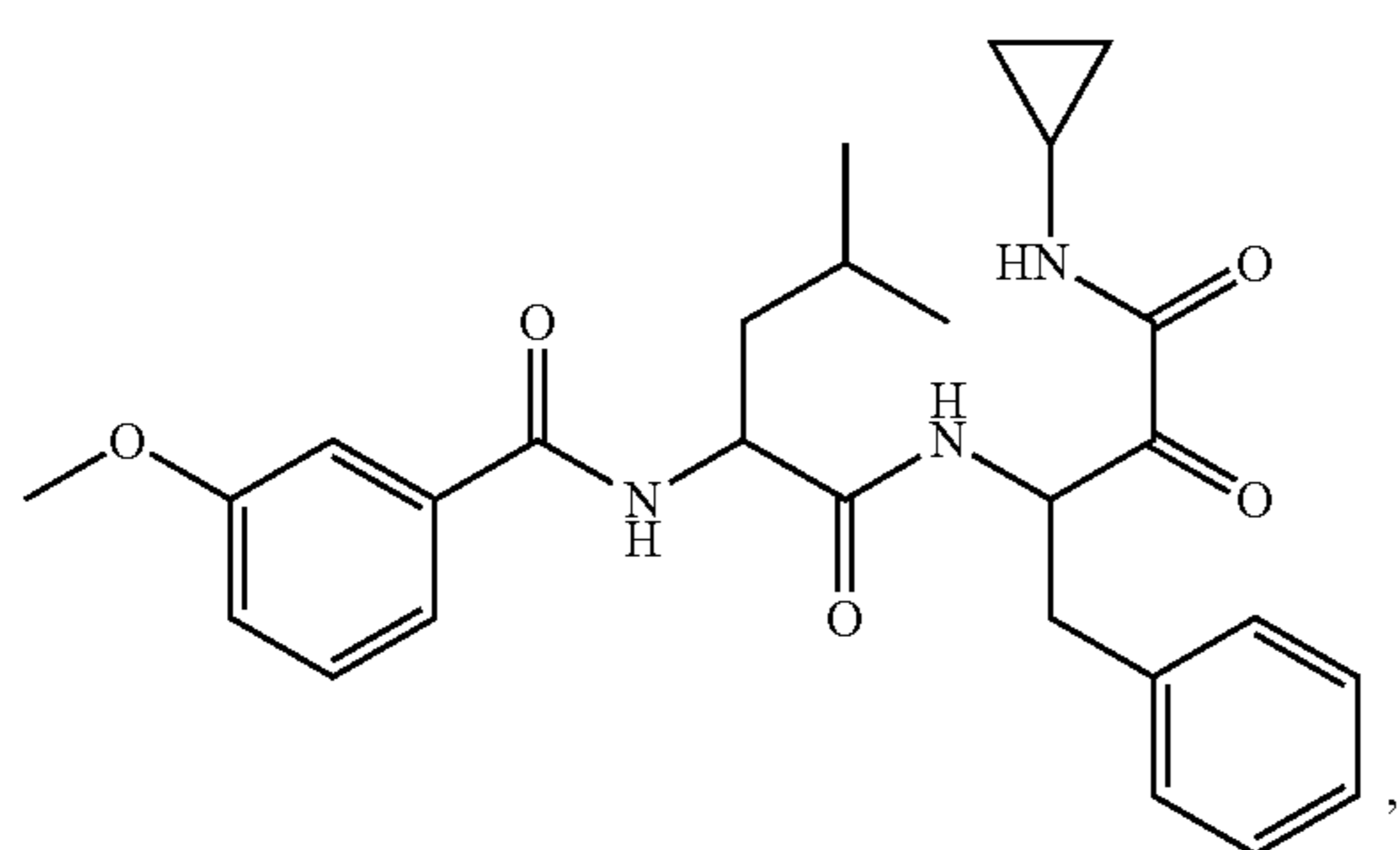
wherein R_{1a} , R_{2a} and R_{3a} have the same meaning as in the compounds of formula (II), or a stereoisomer or a pharmaceutically acceptable salt thereof.

In a particular embodiment, the specific stereoisomer of the compound of formula (II) is a compound of formula (IIb):



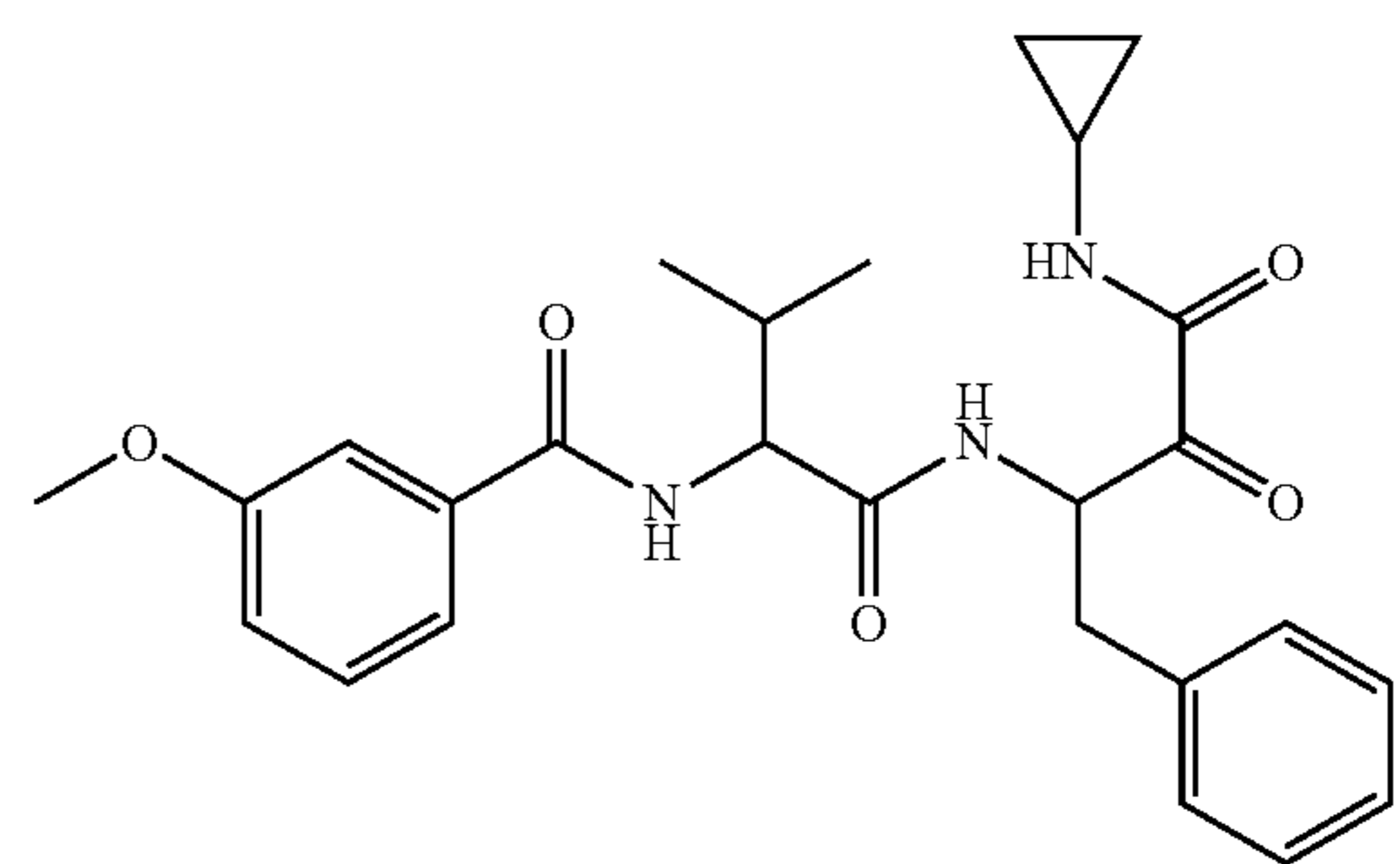
wherein R_{1a} , R_{2a} and R_{3a} have the same meanings as defined for the compounds of formula (II), or a pharmaceutically acceptable salt thereof.

In a preferred embodiment, the compound of formula (II) is selected from the group consisting of:

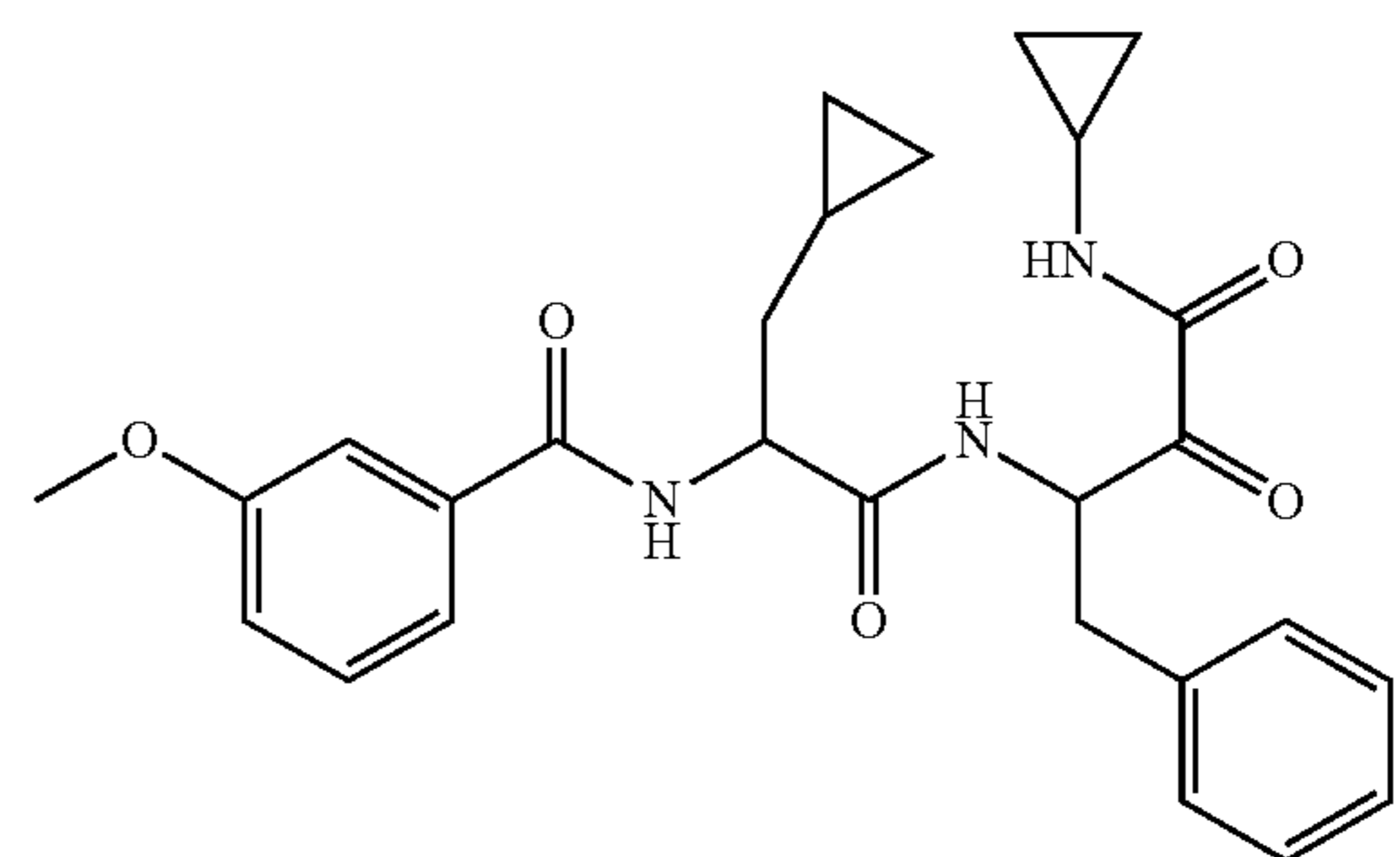


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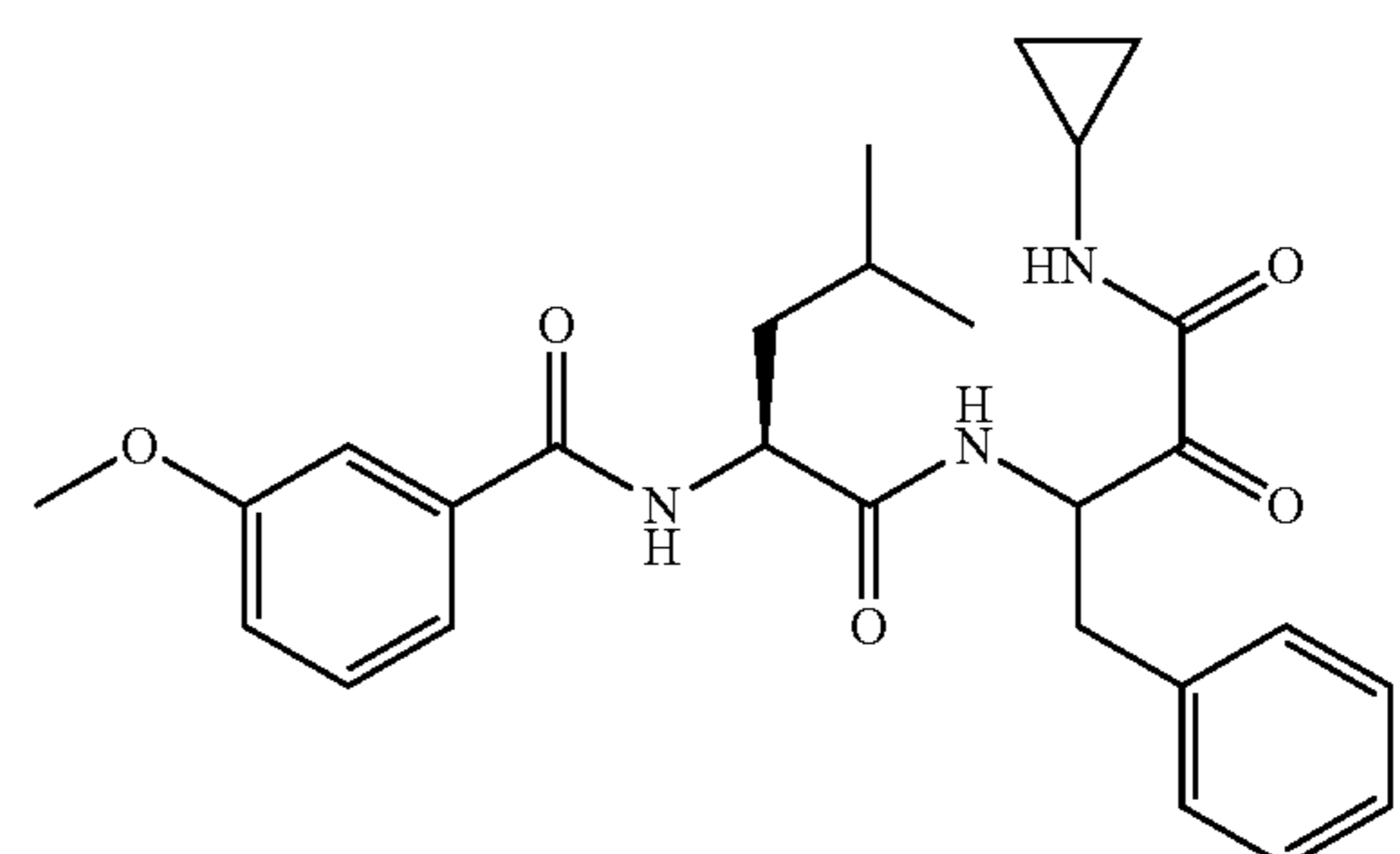


and



or a stereoisomer or pharmaceutically acceptable salt thereof.

In a particular embodiment, the compound of formula (IIa) is selected from the group consisting of:



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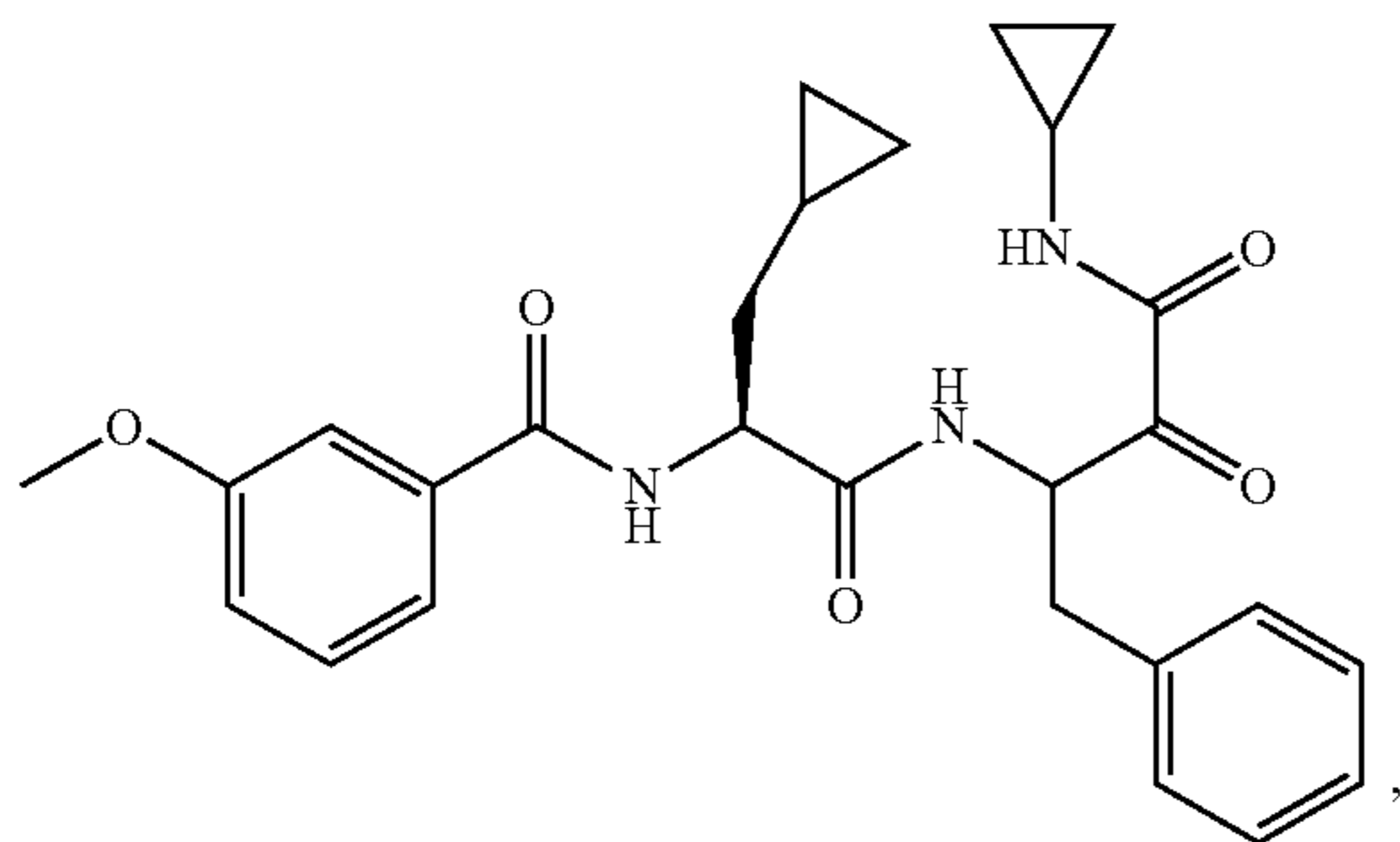
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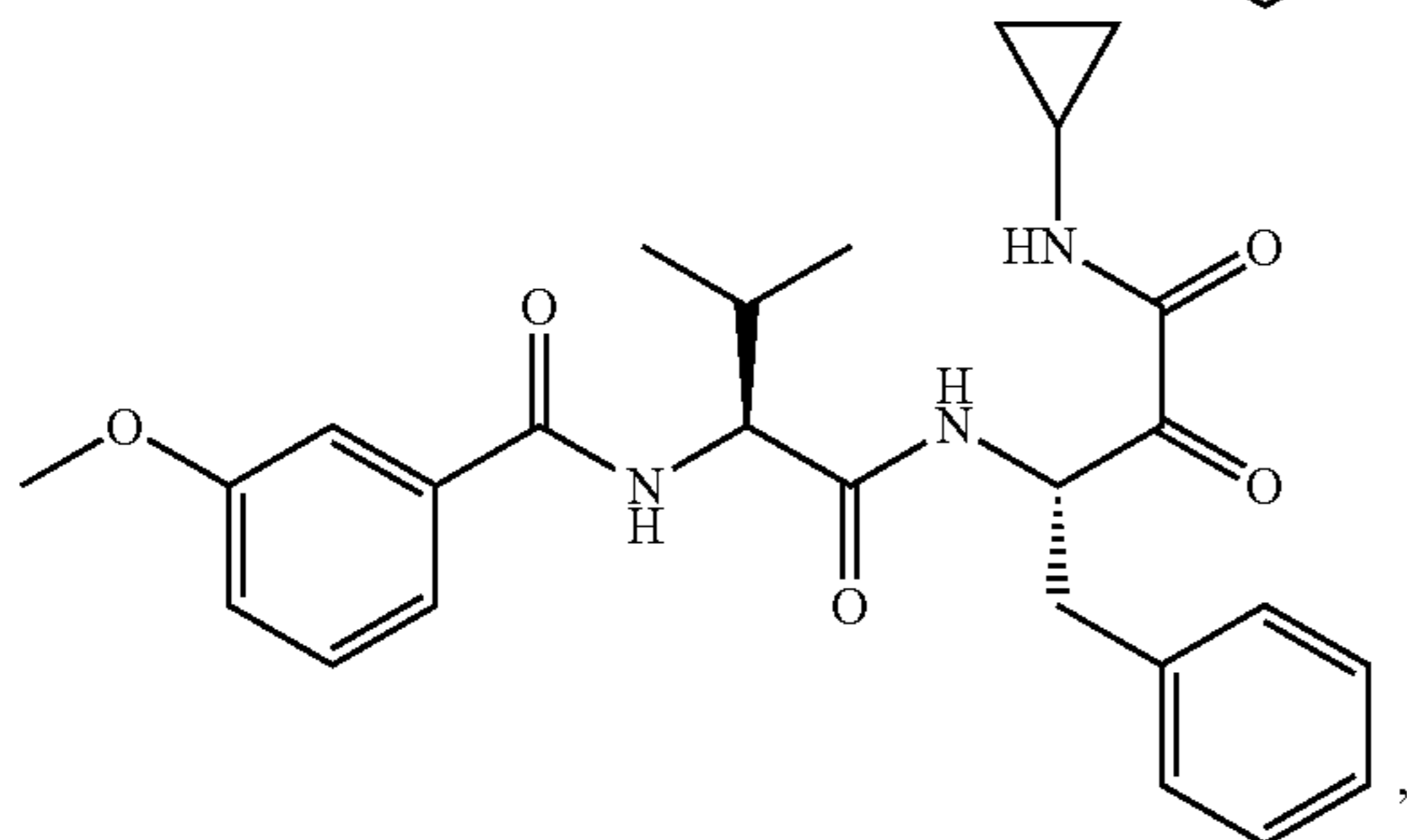
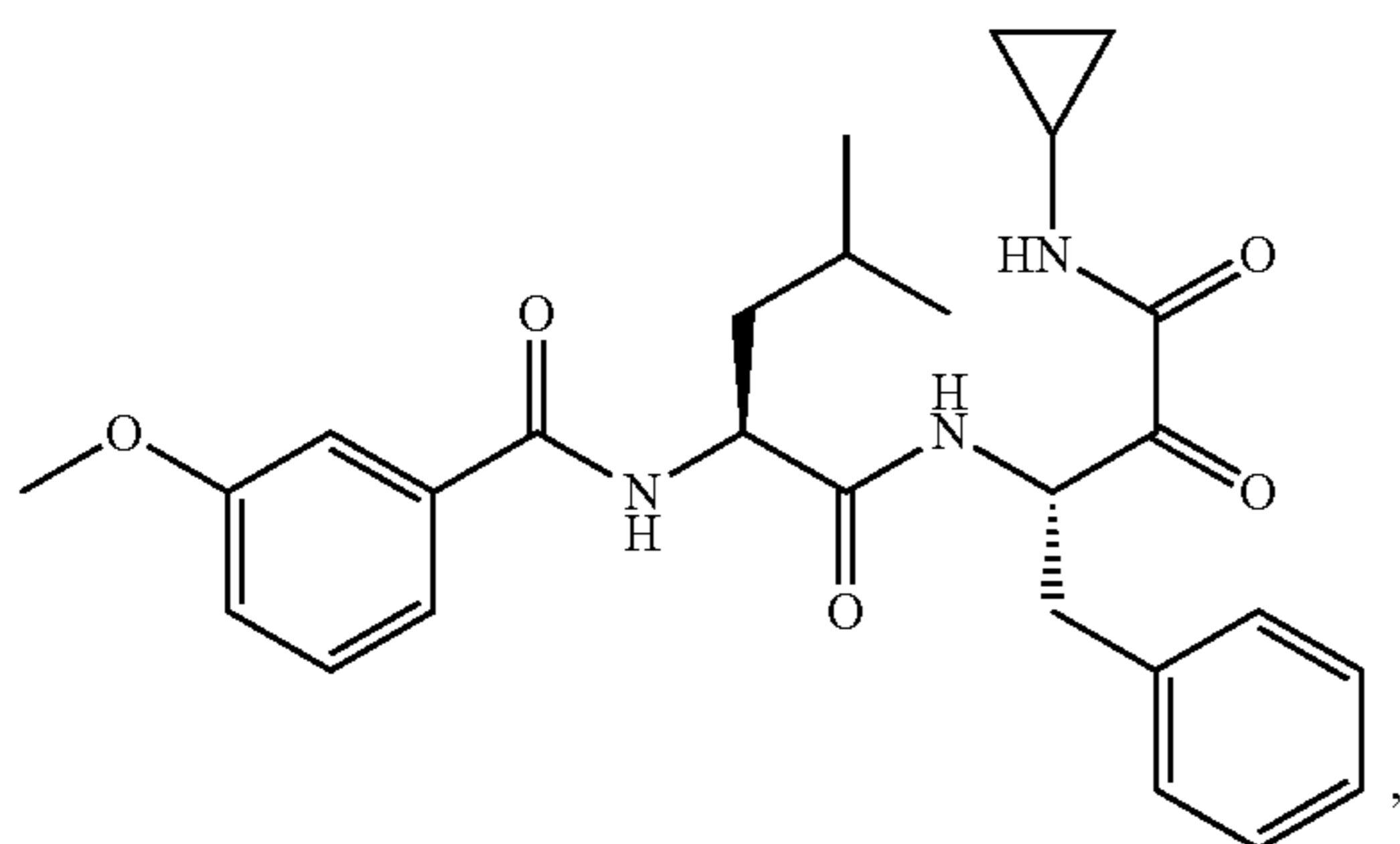
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and

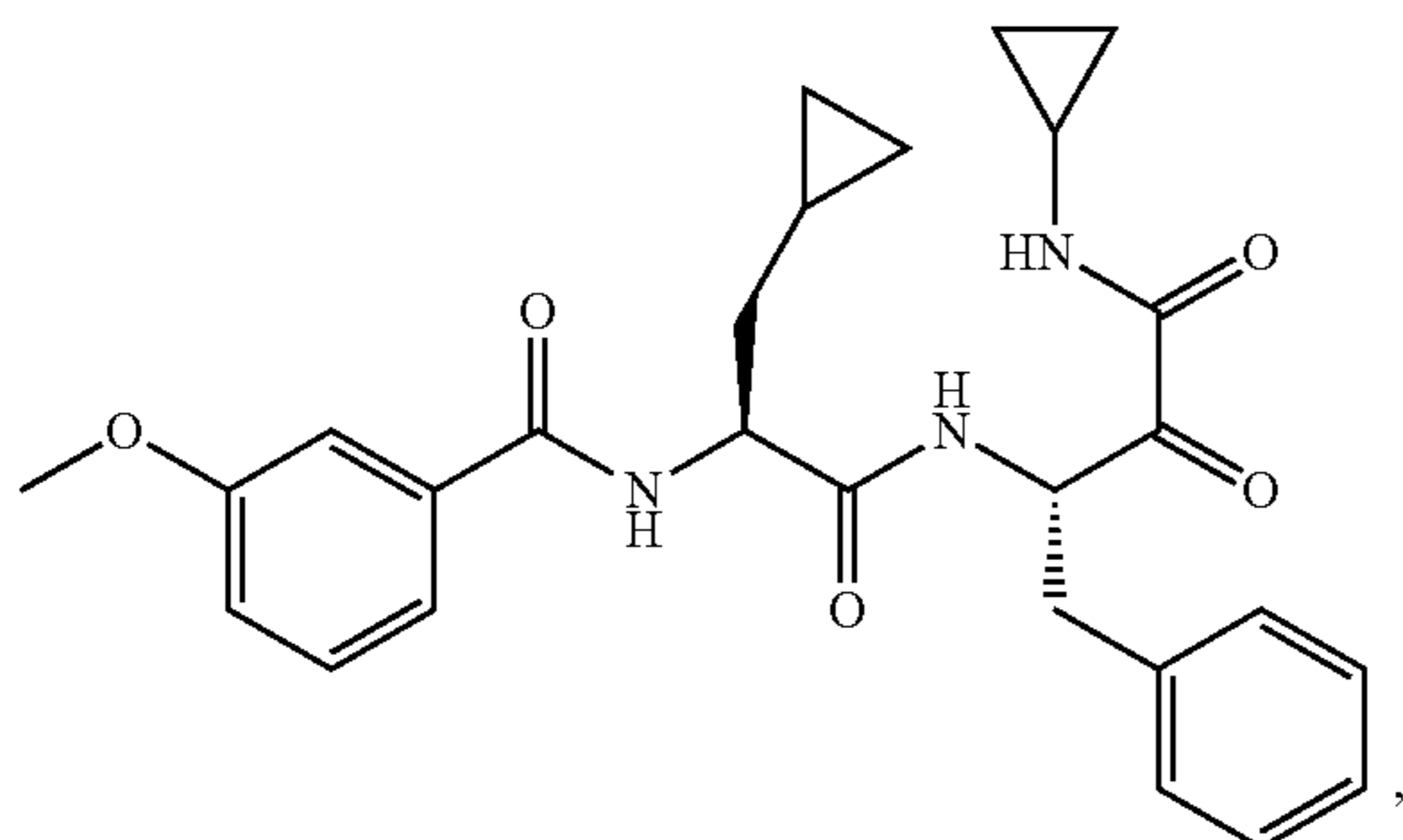


or a stereoisomer or pharmaceutically acceptable salt thereof.

In a particular embodiment, the compound of formula (IIb) is selected from the group consisting of



and



or a pharmaceutically acceptable salt thereof.

Pharmaceutical Compositions

In another aspect, the present invention provides a pharmaceutical composition comprising a compound of formula (II) as defined above, or a stereoisomer or pharmaceutically acceptable salt thereof, and one or more pharmaceutically acceptable excipients.

The term "pharmaceutically acceptable excipient" refers to a vehicle, diluent, or adjuvant that is administered with the

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active ingredient. Such pharmaceutical excipients can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable, or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil, and similars.

5 Water or saline aqueous solutions and aqueous dextrose and glycerol solutions, particularly for injectable solutions, are preferably used as vehicles. Suitable pharmaceutical vehicles are described in "Remington's Pharmaceutical Sciences" by E. W. Martin, 21st Edition, 2005.

10 Compounds of formula (II) of the invention may be administered by the oral, sublingual, parenteral, subcutaneous, intramuscular, intravenous, transdermal, intranasal, intraocular, and/or rectal routes. The compounds may be administered alone or in combination with one or more other

15 compounds of the invention or one or more other drugs.

The pharmaceutical compositions of the present invention may comprise the compounds of formula (II) within liposomes or microvesicles, and may be in the form of dispersions, solutions, lotions, gels, and the like, including topical

20 preparations.

The above definitions do also apply to pharmaceutical compositions comprising a compound of formula (I).

Use of Compounds of Formula (II)

As explained above, the compounds of formula (II) are a

25 subgroup of the compounds of formula (I). Thus, the compounds of formula (II) are also inhibitors of calpain-1. Accordingly, in a fourth aspect, the present invention relates to compounds of formula (II) for use in medicine, in particular for the treatment and/or prevention of diseases or

30 conditions associated with an elevated calpain activity. Preferred diseases or conditions are those selected from the group consisting of heart injury caused by infarction, ischemia with or without reperfusion; neurodegenerative disorders; malaria; diabetic nephropathy; neurotoxicity induced

35 by HIV virus; and cancer such as heart injury caused by infarction, ischemia with or without reperfusion; neurodegenerative disorders; malaria; diabetic nephropathy; neurotoxicity induced by HIV virus; cancer; and fibrotic diseases; a more preferred disease or condition is heart injury caused

40 by infarction, ischemia with or without reperfusion, preferably remodeling after myocardial infarction.

In another aspect, the invention relates to the use of a compound of formula (II) as defined above, or pharmaceutically acceptable salt or stereoisomer thereof, for the manu-

45 facture of a medicament for the treatment and/or prevention of a disease or condition associated with an elevated calpain activity; preferably selected from the group consisting of heart injury caused by infarction, ischemia with or without reperfusion; neurodegenerative disorders; malaria; diabetic

50 nephropathy; neurotoxicity induced by HIV virus; cancer; and fibrotic diseases; more preferably selected from the group consisting of heart injury caused by infarction, ischemia with or without reperfusion, still more preferably remodeling after myocardial infarction.

55 In a further aspect, the present invention relates to a method of treatment and/or prevention a disease or condition associated with an elevated calpain activity which comprises administering to a subject in need thereof a therapeutically effective amount of a compound of formula (II) as defined

60 above or a pharmaceutically acceptable salt or stereoisomer thereof. Preferably the disease or condition associated with an elevated calpain activity is selected from the group consisting of heart injury caused by infarction, ischemia with or without reperfusion; neurodegenerative disorders;

65 malaria; diabetic nephropathy; neurotoxicity induced by HIV virus; cancer; and fibrotic diseases; more preferably selected from the group consisting of heart injury caused by

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infarction, ischemia with or without reperfusion, still more preferably remodeling after myocardial infarction.

In particular embodiments, the disease or condition is selected from the group consisting of heart injury caused by infarction, ischemia with or without reperfusion; neurodegenerative disorders; malaria; diabetic nephropathy; neurotoxicity induced by HIV virus; and cancer.

In other particular embodiments, the disease or condition is heart injury caused by infarction, ischemia with or without reperfusion, preferably remodeling after myocardial infarction.

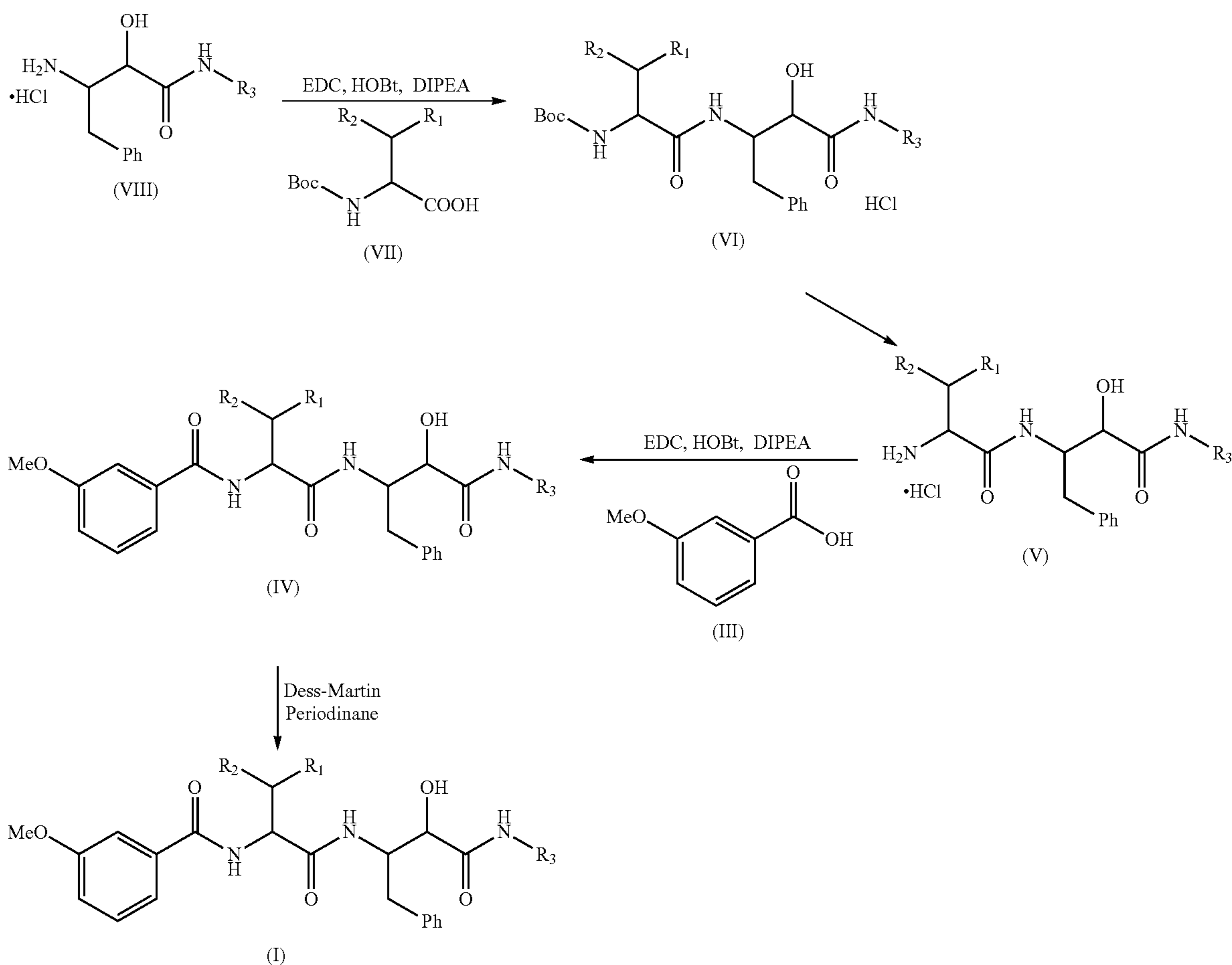
In other particular embodiments, the neurodegenerative disorder is selected from the group consisting of Alzheimer's disease, Parkinson disease, multiple sclerosis, acute autoimmune encephalitis, and Creutzfeldt-Jakob disease.

In other particular embodiments, the cancer is selected from the group consisting of breast cancer, colorectal cancer and leukaemia.

In other particular embodiments, the fibrotic disease is selected from the group consisting of cardiac fibrosis, lung fibrosis, liver fibrosis, renal fibrosis and retroperitoneal fibrosis.

Process for the Preparation of Compounds of Formulae (I) and (II)

The compounds of formula (I) and (II), preferably wherein R_3 is a cycloalkyl group, may be prepared starting from the N-substituted 3-amino-2-hydroxy-amide hydrochlorides of formula (VIII) following the synthetic scheme shown below:

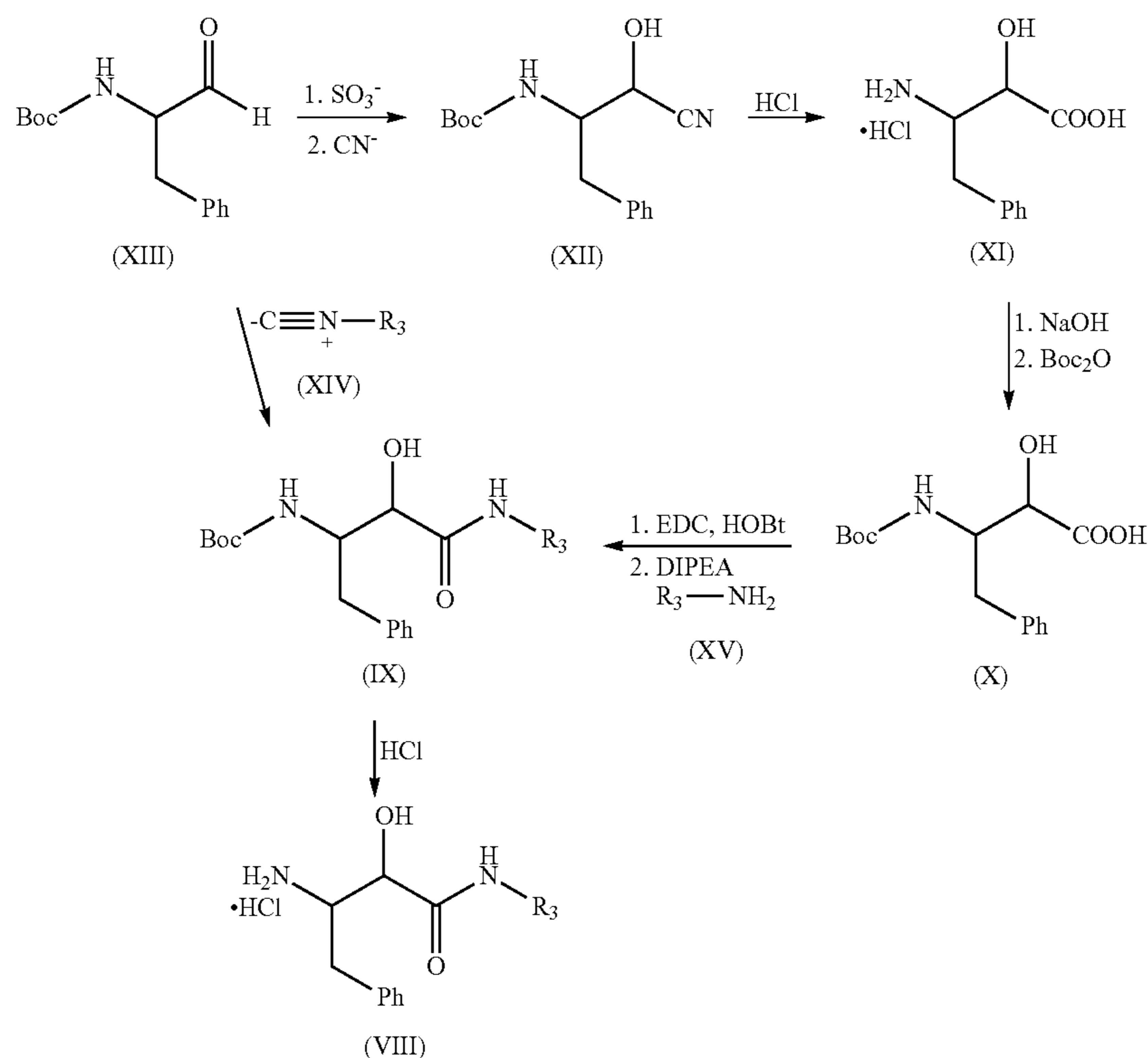


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In a first step the protected amino acid of formula (VII), HOBt (hydroxybenzotriazol), EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide) and the N-substituted 3-amino-2-hydroxy-amide hydrochlorides of formula (VIII) are dissolved in a solvent such as dichloromethane (DCM). DIPEA (N,N-diisopropylethylamine) is then added and the mixture is left to react to yield the compound of formula (VI). Other amide coupling agents are equally effective such as HATU (1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate) in the presence of DIPEA in dimethylformamide (DMF) or T3P (propylphosphonic anhydride) in the presence of NEt_3 in DMF. The compound of formula (VI) was reacted in a solvent such as 1,4-dioxane with a strong acid such as hydrochloric acid or in DCM and treated with trifluoroacetic acid (TFA) to yield the compound of formula (V). The compound of formula (III), HOBt, EDC and the compound of formula (V) are dissolved in a solvent such as DCM. DIPEA is then added and the mixture is left to react to yield the compound of formula (IV). Other amide coupling agents are equally effective such as HATU in the presence of DIPEA in DMF or T3P in the presence of NEt_3 in DMF. Finally, the compound of formula (IV) is dissolved in solvents such as DCM, DMF or a mixture of them and Dess-Martin periodinane is added to yield the compound of formula (I). Other oxidants such as DCC (N,N'-dicyclohexylcarbodiimide) in DMSO (dimethylsulfoxide) are equally useful.

The N-substituted 3-amino-2-hydroxy-amide hydrochlorides of formula (VIII) may be obtained starting from the protected aminoaldehydes of formula (XIII) following the synthetic scheme shown below:

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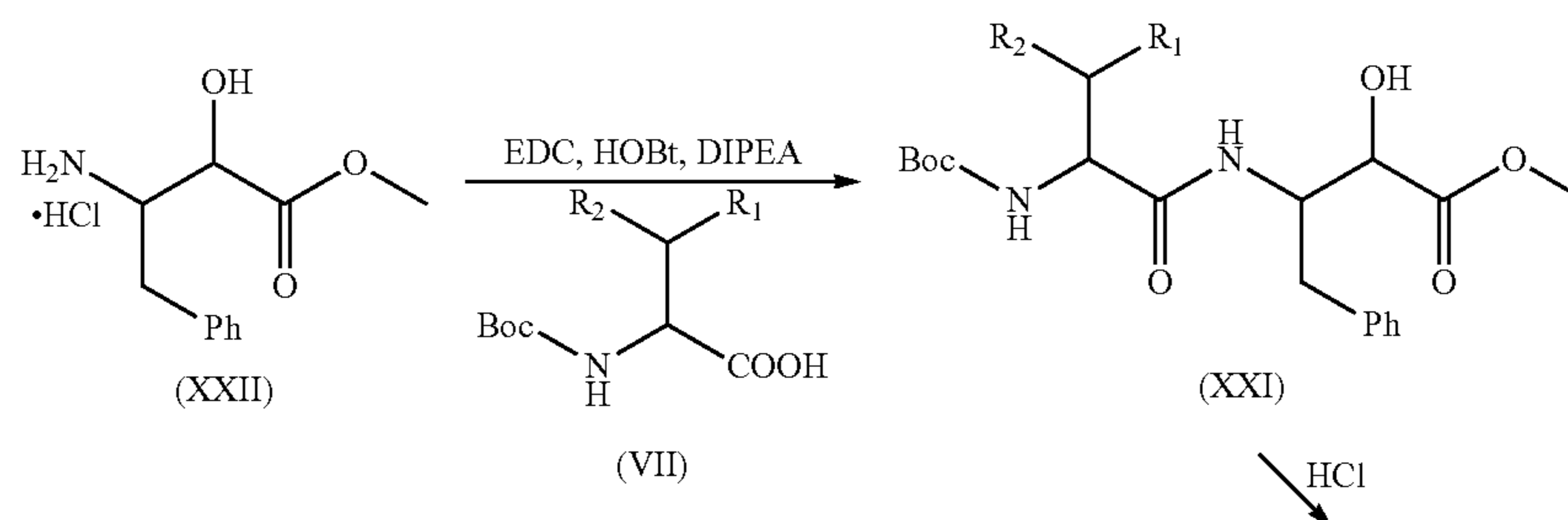


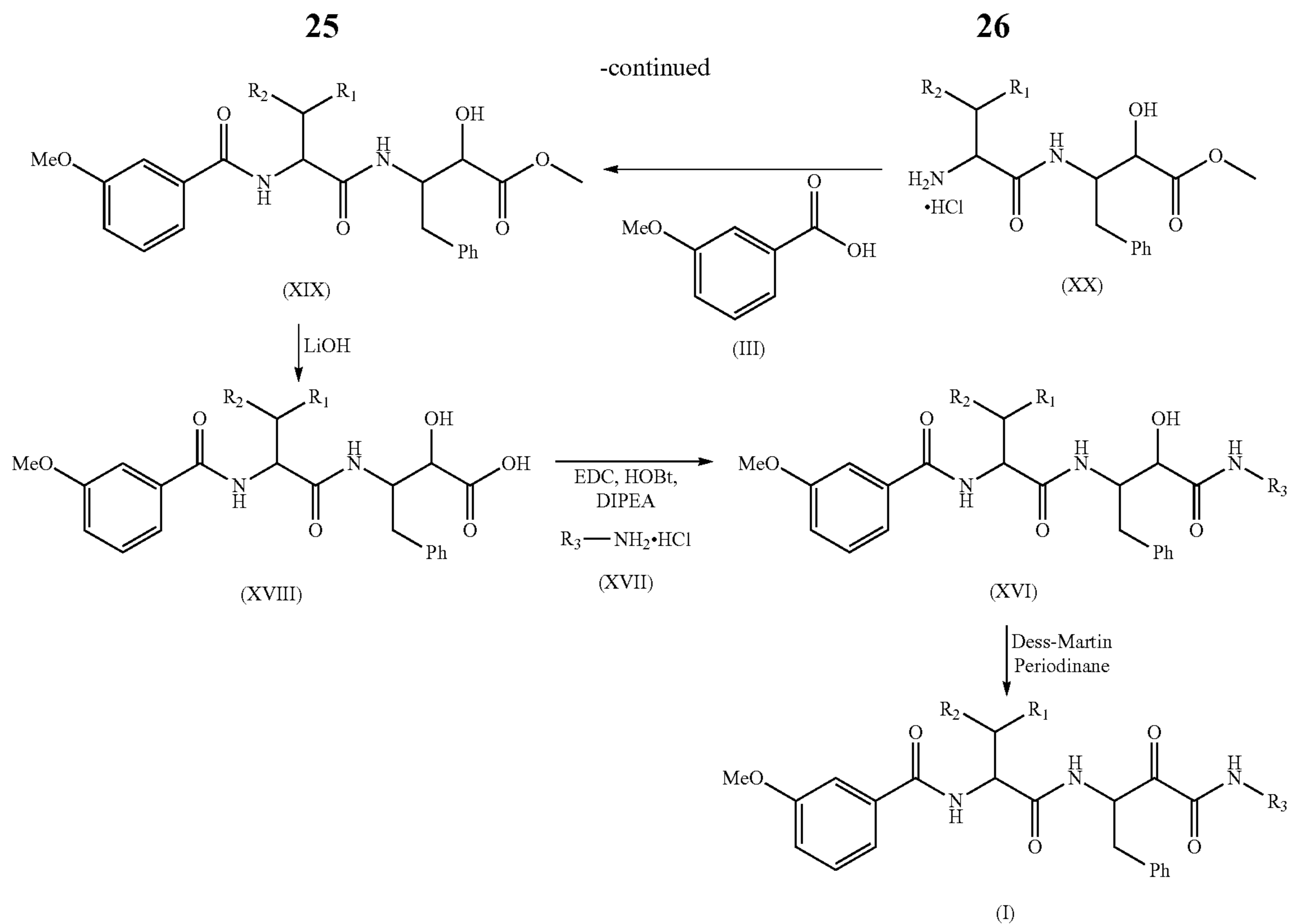
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In a first step the protected aminoaldehydes of formula (XIII) are dissolved in a solvent such as 1,4-dioxane and sodium bisulfite is added followed by the addition of a potassium cyanide aqueous solution to yield the compound of formula (XII). The compound of formula (XII) is dissolved in a concentrated acid aqueous solution such as concentrated hydrochloric acid and refluxed to yield the compound of formula (XI). An aqueous solution of compound of formula (XI) is brought to alkaline pH (preferably in the range of 10-12) for example with sodium hydroxide and Boc_2O (di-tert-butyl dicarbonate) is added. After total conversion the mixture is acidified for example with KHSO_4 and the compound of formula (X) is extracted with a water-immiscible solvent such as ethyl acetate. The compound of formula (X), HOBt and EDC are dissolved in a solvent such as anhydrous DCM. DIPEA and the amine of formula (XV) are then added and the mixture is left to react for 8 to 24 hours react to yield the compound of formula (VIII). T3P or HATU can also be used instead of EDC and HOBt with good results. The compound of formula (IX) was reacted in a solvent such as 1,4-dioxane with a strong acid such as hydrochloric acid to yield the compound of formula (VIII).

In an alternative synthetic path which is also illustrated in the previous scheme, the protected aminoaldehydes of formula (XIII) are dissolved in a solvent such as anhydrous DCM and an acid such as acetic acid and an isocyanide compound of formula (XIV) are added and left to react in an inert atmosphere such as argon atmosphere at room temperature. Then solvent is removed and the resulting compound is extracted with ethyl acetate and washed with a saturated sodium bicarbonate aqueous solution. The product was then solved in a mixture of THF (tetrahydrofuran) and MeOH (methanol) and treated with a lithium hydroxide aqueous solution to yield the compound of formula (IX). Then, the compound of formula (IX) is reacted, as described above, in a solvent such as 1,4-dioxane with a strong acid such as hydrochloric acid to yield the compound of formula (VIII).

The compounds of formula (I) wherein R_3 is an alkoxy group may be prepared starting from the N-substituted 3-amino-2-hydroxy-amide hydrochlorides of formula (VIII) following the synthetic scheme shown below:

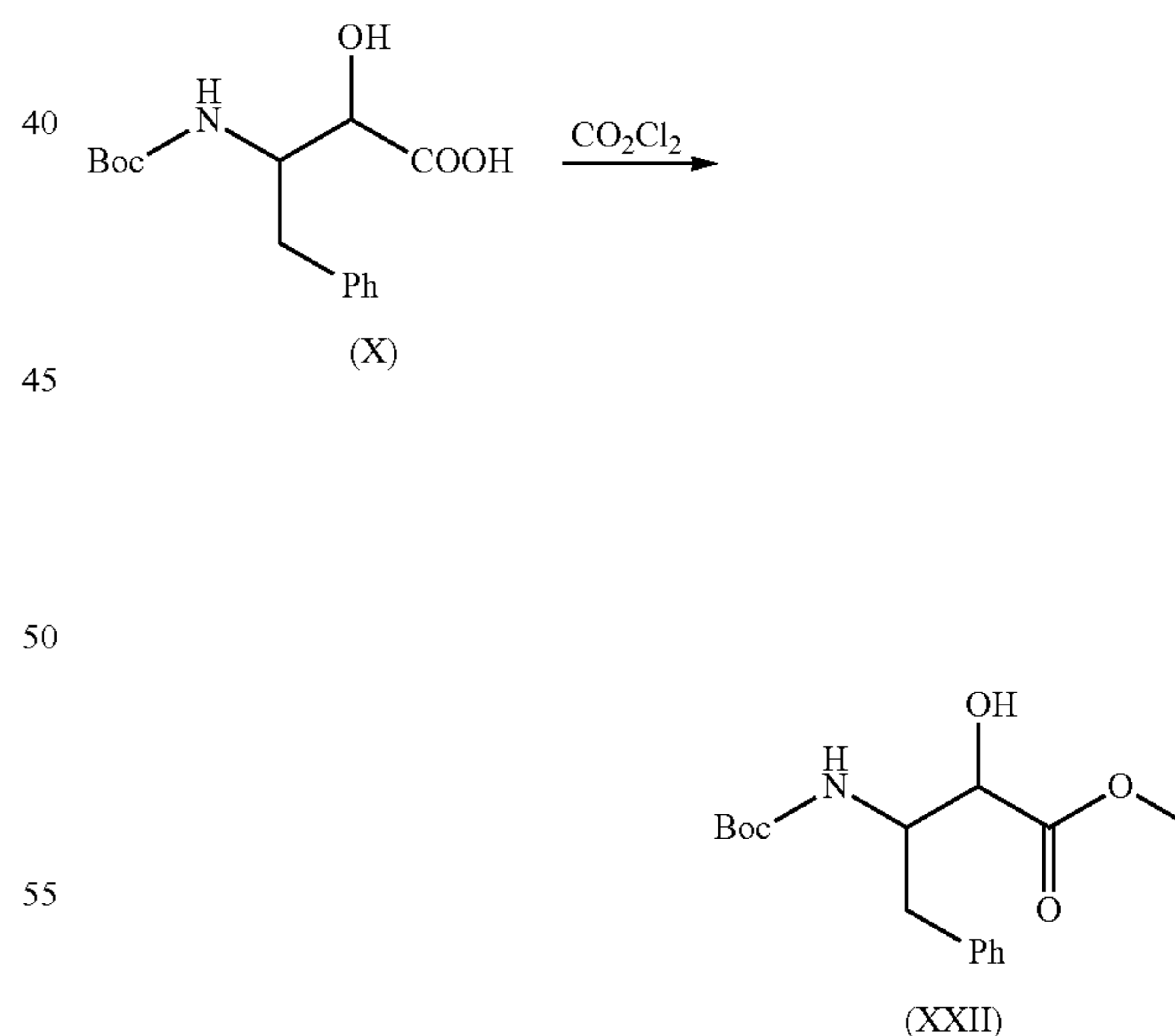




In a first step the protected amino acid of formula (VII), HOBt, and the compound of formula (XXII) are dissolved in a solvent such as dichloromethane (DCM). DIPEA is then added and the mixture is left to react to yield the compound of formula (XXI). Other amide coupling agents are equally effective such as HATU in the presence of DIPEA in DMF or T3P in the presence of NEt_3 in DMF. The compound of formula (XXI) was reacted in a solvent such as 1,4-dioxane with a strong acid such as hydrochloric acid or in DCM and treated with trifluoroacetic acid (TFA) to yield the compound of formula (XX). The compound of formula (III), HOBt, EDC and the compound of formula (XX) are dissolved in a solvent such as DCM. DIPEA is then added and the mixture is left to react to yield the compound of formula (XIX). Other amide coupling agents are equally effective such as HATU in the presence of DIPEA in DMF or T3P in the presence of NEt_3 in DMF. The compound of formula (XIX) was then treated with LiOH in a mixture of THF/MeOH/water and subsequently treated with an aqueous acid solution such as HCl to give a compound of formula (XVIII). The compound of formula (XVII), HOBt, EDC and the compound of formula (XVIII) are dissolved in a solvent such as DCM. DIPEA is then added and the mixture is left to react to yield the compound of formula (XVI). Other amide coupling agents are equally effective such as HATU in the presence of DIPEA in DMF or T3P in the presence of NEt_3 in DMF. Finally, the compound of formula (XVI) is dissolved in solvents such as DCM, DMF or a mixture of them and Dess-Martin periodinane is added to yield the compound of formula (I). Other oxidants such as DCC ($\text{N,N}'$ -dicyclohexylcarbodiimide) in DMSO (dimethylsulfoxide) are equally useful.

The compound of formula (XXII) may be obtained from a compound of formula (X), whose synthesis has been

described above, by treatment with oxalyl chloride in methanol, as shown in the synthetic scheme below:



The starting compounds of formulae (XVII), (XIII), (XIV), (XV), (VII) and (III) are either commercially available or may be obtained by methods described in the literature.

The following examples are merely illustrative of certain embodiments of the invention and cannot be considered as restricting it in any way.

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EXAMPLES

Abbreviations

The following abbreviations are used in the examples:

Boc: tert-butoxycarbonyl

conc: concentrate

Boc₂O: di-tert-butyl dicarbonate

DCM: dichloromethane

DIPEA: N,N-Diisopropylethylamine

DMSO: dimethylsulfoxide

EDC HCl: 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride

EtOAc: ethyl acetate

HBTU: N,N,N',N'-Tetramethyl-O-(1H-benzotriazol-1-yl)uronium hexafluorophosphate

HCl: Hydrochloric Acid

HEPES: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

HOBt: hydroxybenzotriazol

LC-MS: liquid chromatography-mass spectrometry

Leu-OH: Leucine

MeOH: methanol

Min: minutes

Phe-OH: phenylalanine

Sat: saturated

T3P=Propylphosphonic Anhydride

TBME: tert-butyl methyl ether

THF: tetrahydrofuran

t_R: retention time

Val-OH: valine

Materials and Methods

LC-MS: The products of the examples were characterized using liquid chromatography coupled to mass spectroscopy (LC-MS). HPLC-MS analysis was carried out using the following procedure: In a an Alliance HT 2795 (Waters) chromatograph equipped with 2996 photodiode array detector and coupled to mass 3100 detector LC/MS. Separation was achieved using a XBridge C₁₈ column (50×4.6 mm, S-3.5 μm) and using mixtures of a 10 mM NH₄CO₃ aqueous solution of pH=9 (A) and acetonitrile (B) as eluents at 50° C. and 1.6 mL/min flow rate using the following eluting conditions: 5% to 100% B in 3.5 min. The detector was set at electrospray positive mode (ESI+) in the mass range of 100-700. Cone voltage 10 V. Source T: 120° C. Desolvation T: 350° C.

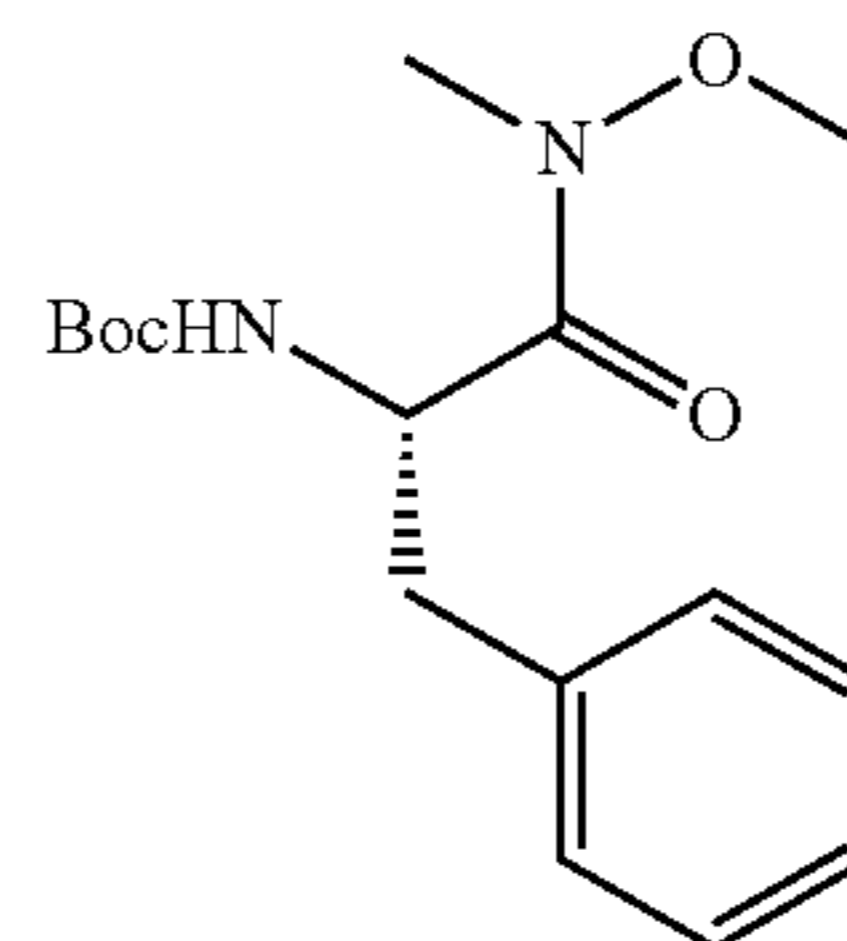
Reagents: 2-methoxybenzoic acid (Sigma Aldrich); 3-methoxybenzoic acid (Sigma Aldrich); 4-methoxybenzoic acid (Sigma Aldrich); Acetic acid (VWR); Boc-L-Phe-OH (Fluorochem); Boc-L-Leu-OH (Fluorochem); Boc-L-Val-OH (Fluorochem); cyclopropylamine (Sigma Aldrich); cyclopropylisocyanide (Fluorochem); Dess-Martin Periodinane 15% solution in DCM (Acros); EDC (Iris Biotech); HBTU (Iris Biotech); HCl 4N in 1,4-Dioxane (TCI Europe Organic Chem); HOBt (Carbosynth); LiOH.H₂O (Sigma Aldrich); Lithium aluminium hydride solution 1.0 M in tetrahydrofuran (Sigma Aldrich); N,O-dimethylhydroxylamine hydrochloride (Fluorochem); (S)-2-(Boc-amino)-3-cyclopropylpropanoic acid (Fluorochem).

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Solvents: DCM (Scharlab); Ethanol (Panreac); EtOAc (Scharlab); Hexane (Scharlab); MeOH (Scharlab); TBME (SDS); THF (Panreac).

Synthesis of Intermediates

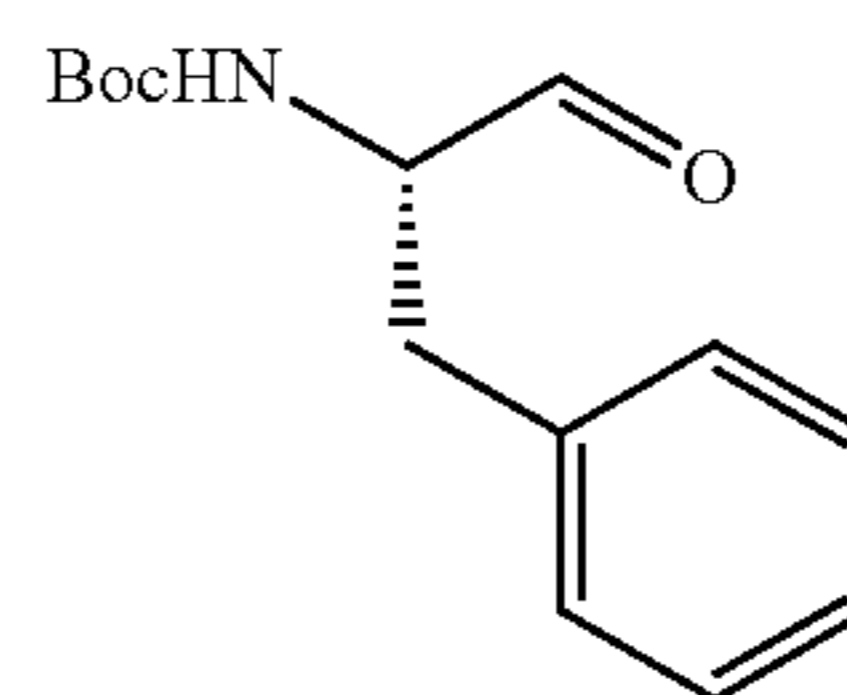
Intermediate 1: (S)-tert-butyl(1-(methoxy(methyl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate



To a solution of Boc-L-Phe-OH (10.0 g, 37.7 mmol, 1.0 eq.) in DCM (150 ml), was added N,O-dimethylhydroxylamine hydrochloride (4.0 g, 41.1 mmol, 1.1 eq), HBTU (III) (15.7 g, 41.1 mmol, 1.1 eq) and DIPEA (20 ml, 113.0 mmol, 3.0 eq). The reaction mixture was stirred for 1 hour at room temperature. Volatiles were then removed under vacuum and the crude mixture extracted with EtOAc washed with a saturated solution of NaHCO₃ (2×200 mL). The crude material was purified by flash chromatography (ISCO Rf) using Hexane/EtOAc as eluents, from 0% to 40% in EtOAc, the product eluted at 35%. 11.6 g were obtained (37.7 mmol, 100% yield).

LC-MS: t_R=2.58 min; m/z=309

Intermediate 2: Synthesis of (S)-tert-butyl (1-oxo-3-phenylpropan-2-yl)carbamate

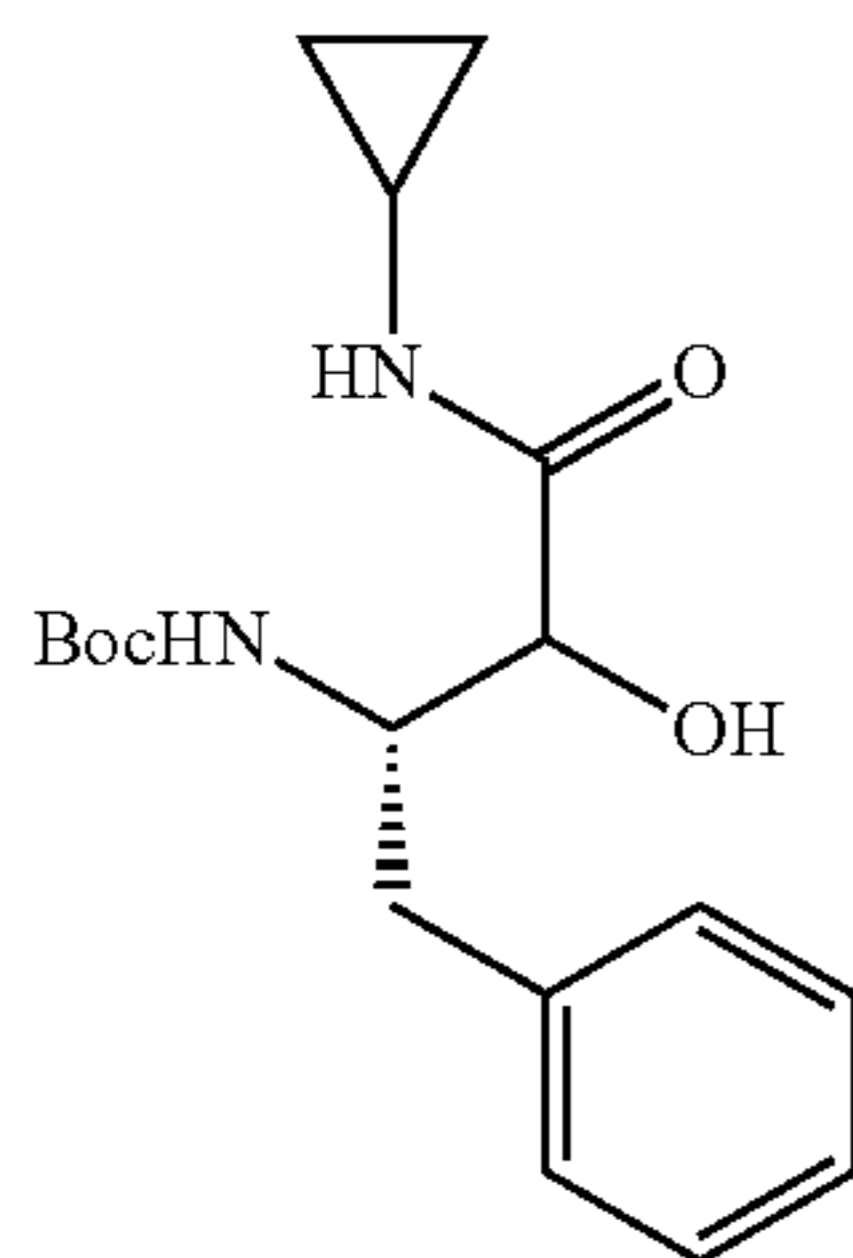


Lithium aluminium hydride solution 1.0 M in tetrahydrofuran (37.7 ml, 37.7 mmol, 1.0 eq) was added to a solution of Intermediate 1 (11.6 g, 37.7 mmol, 1.0 eq.) in THF (250 ml) cooled to 0° C. The mixture was stirred overnight to room temperature. The reaction mixture was diluted with EtOAc and solvent was evaporated. The crude material was purified by silica gel chromatography using Hexane/EtOAc as solvents, from 0% to 60% in EtOAc, product eluted at 25%. 6.7 g of the desire product was obtained (26.9 mmol, 68% yield).

LC-MS: t_R=2.48 min; m/z=250

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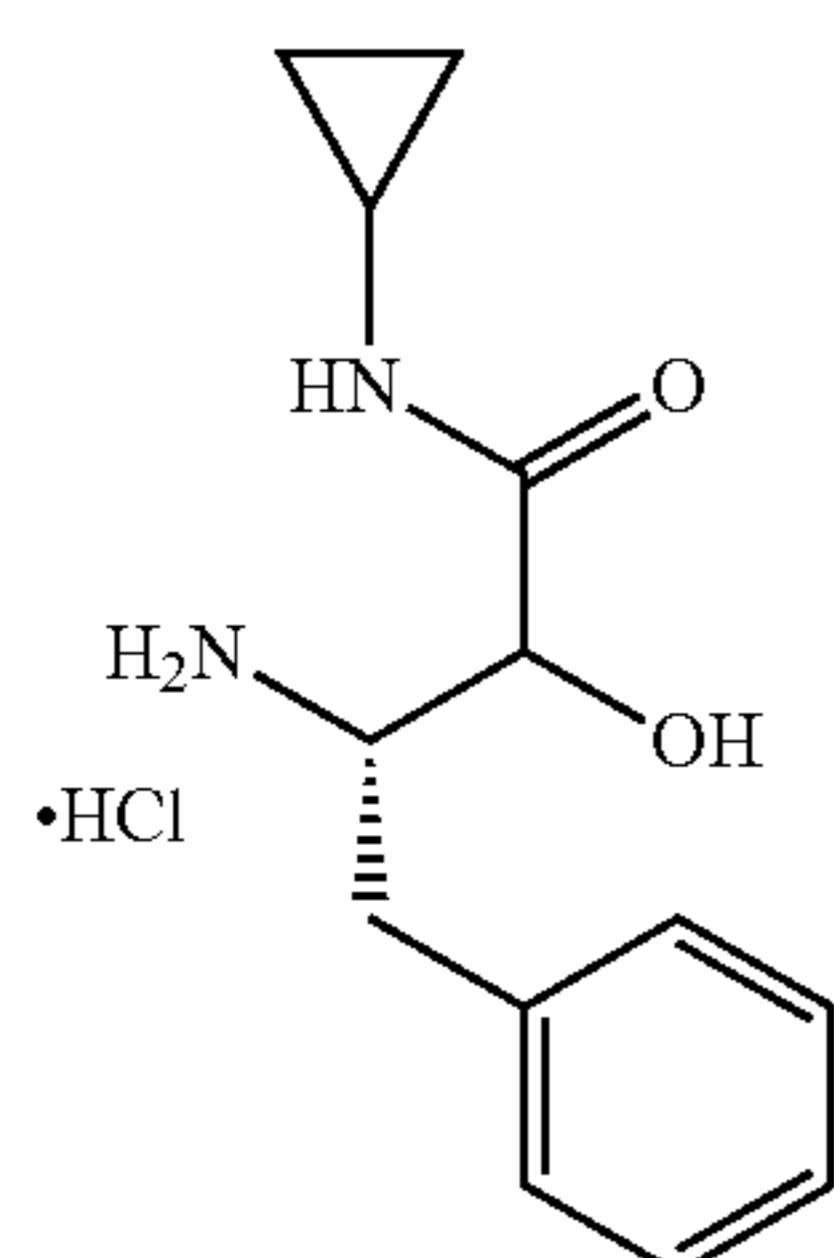
Intermediate 3: Synthesis of tert-butyl ((2S)-4-(cyclopropylamino)-3-hydroxy-4-oxo-1-phenylbutan-2-yl)carbamate



Acetic acid (0.35 ml, 8.0 mmol, 2.0 eq.) and cyclopropyl isocyanide (0.30 ml, 4.4 mmol, 1.1 eq.) were added to a solution of intermediate 2 (1.0 g, 4.0 mmol, 1.0 eq) in DCM (20 ml), and the mixture was stirred at rt. After 15 min, a mixture of THF/MeOH/H₂O (7/5/3) was added to the mixture and followed by LiOH.H₂O (0.67 g, 16.0 mmol, 4 eq.). After 15 min, the solvents were removed under vacuum. The product was extracted into EtOAc, and the solution washed with a sat. solution of NaHCO₃ and purified on flash chromatography (ISCO Rf) using Hexane/Hexane:Ethanol (8:2) as solvents, from 0% to 50% in Hexane:Ethanol (8:2), product eluted at 25%. 0.31 g of product was obtained (23% yield).

LC-MS: t_R =2.32 min; m/z =335

Intermediate 4: Synthesis of (3S)-3-amino-N-cyclopropyl-2-hydroxy-4-phenylbutanamide hydrochloride



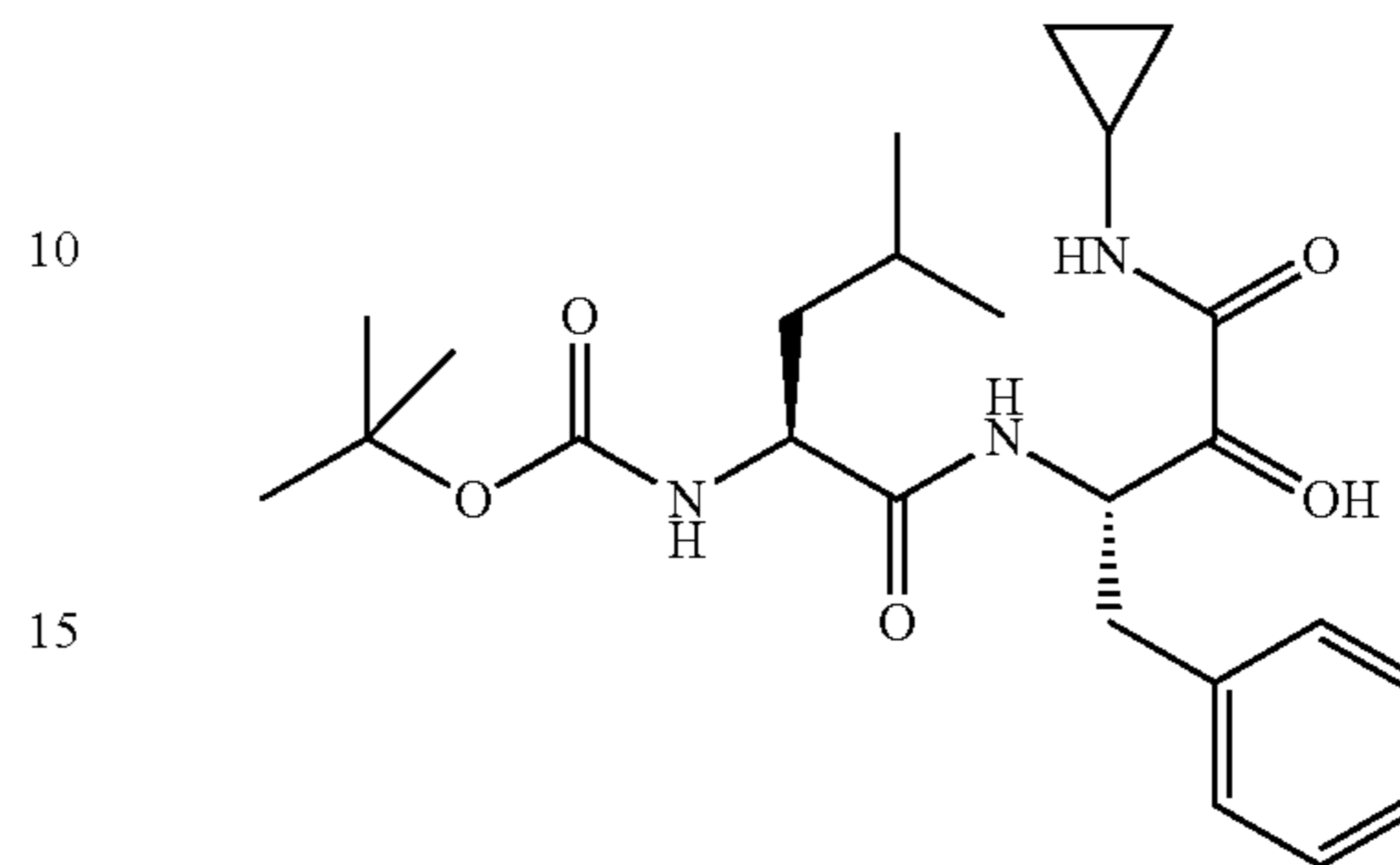
A solution of HCl 4 N in 1,4-dioxane (1 ml, 4.1 mmol, 4 eq.) was added to intermediate 3 (0.31 g, 0.9 mmol, 1.0 eq.). The mixture was stirred at room temperature for 1 h and then evaporated to dryness. The product was used for the next step without further purification.

LC-MS: t_R =1.50 min; m/z =235

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Intermediate 5: tert-butyl ((2S)-1-(((2S)-4-(cyclopropylamino)-3-hydroxy-4-oxo-1-phenylbutan-2-yl)amino)-4-methyl-1-oxopentan-2-yl)carbamate

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A solution of intermediate 4 (251 mg, 0.9 mmol, 1.0 eq.) and DIPEA (0.6 ml, 3.7 mmol, 4 eq.) in DCM (5 mL) was added to a solution of Boc-L-Leu-OH (300 mg, 1.2 mmol, 1.2 eq.), EDC (249 mg, 1.3 mmol, 1.5 eq.) and HOBt (199 mg, 1.3 mmol, 1.5 eq.) in DCM (5 mL). The reaction mixture was stirred for 1 hour at room temperature. The volatiles were removed under vacuum. The crude mixture was extracted with EtOAc and washed with a sat. solution of NaHCO₃ (2×10 mL) and purified by flash chromatography (ISCO Rf) using Hexane/TBME as eluents, from 0% to 80% in TBME, the product eluted at 60%. 298 mg of the product was obtained (0.7 mmol, 72% yield).

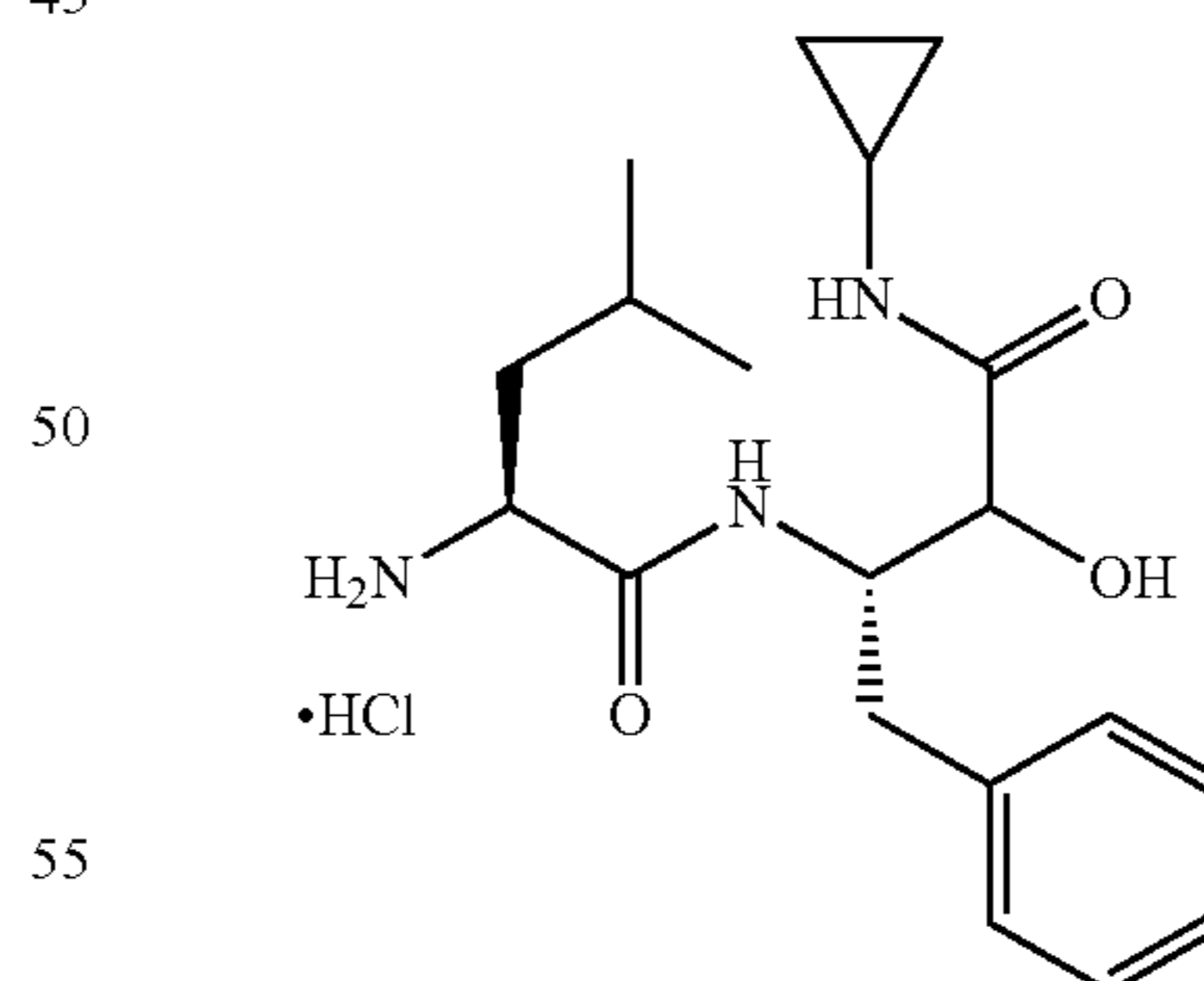
LC-MS (Method A): t_R =2.60 min; m/z =448

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Intermediate 6: (2S)-2-amino-N-((2S)-4-(cyclopropylamino)-3-hydroxy-4-oxo-1-phenylbutan-2-yl)-4-methylpentanamide hydrochloride

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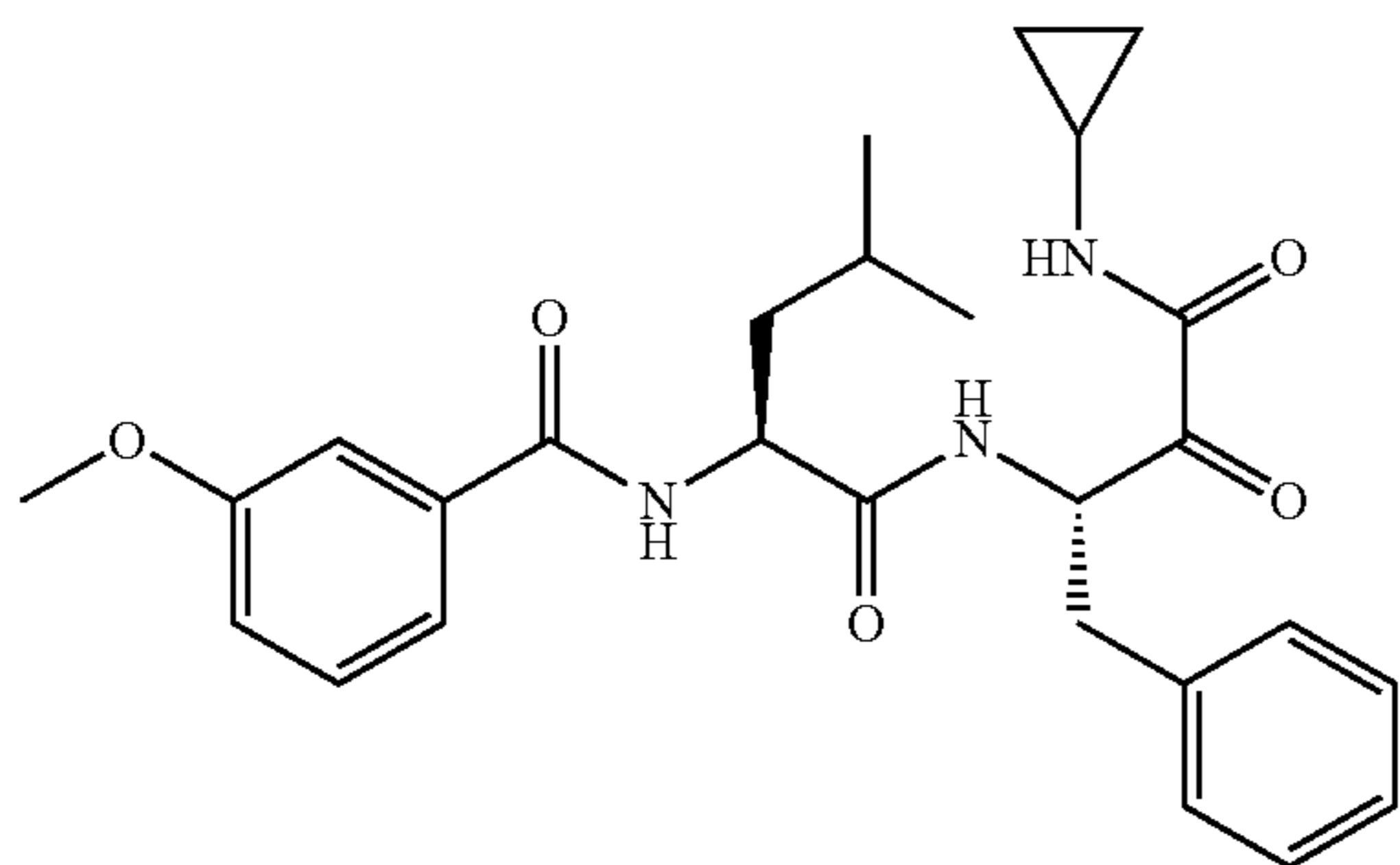
A solution of HCl (4 N) in 1,4-dioxane (3.3 ml, 13.2 mmol, 20 eq.) was added to intermediate 5 (298 mg, 0.6 mmol, 1.0 eq.). The mixture was stirred at room temperature for 1 h and then evaporated to dryness. The product was used for next step without further purification.

LC-MS: t_R =1.92-2.03 min; m/z =348

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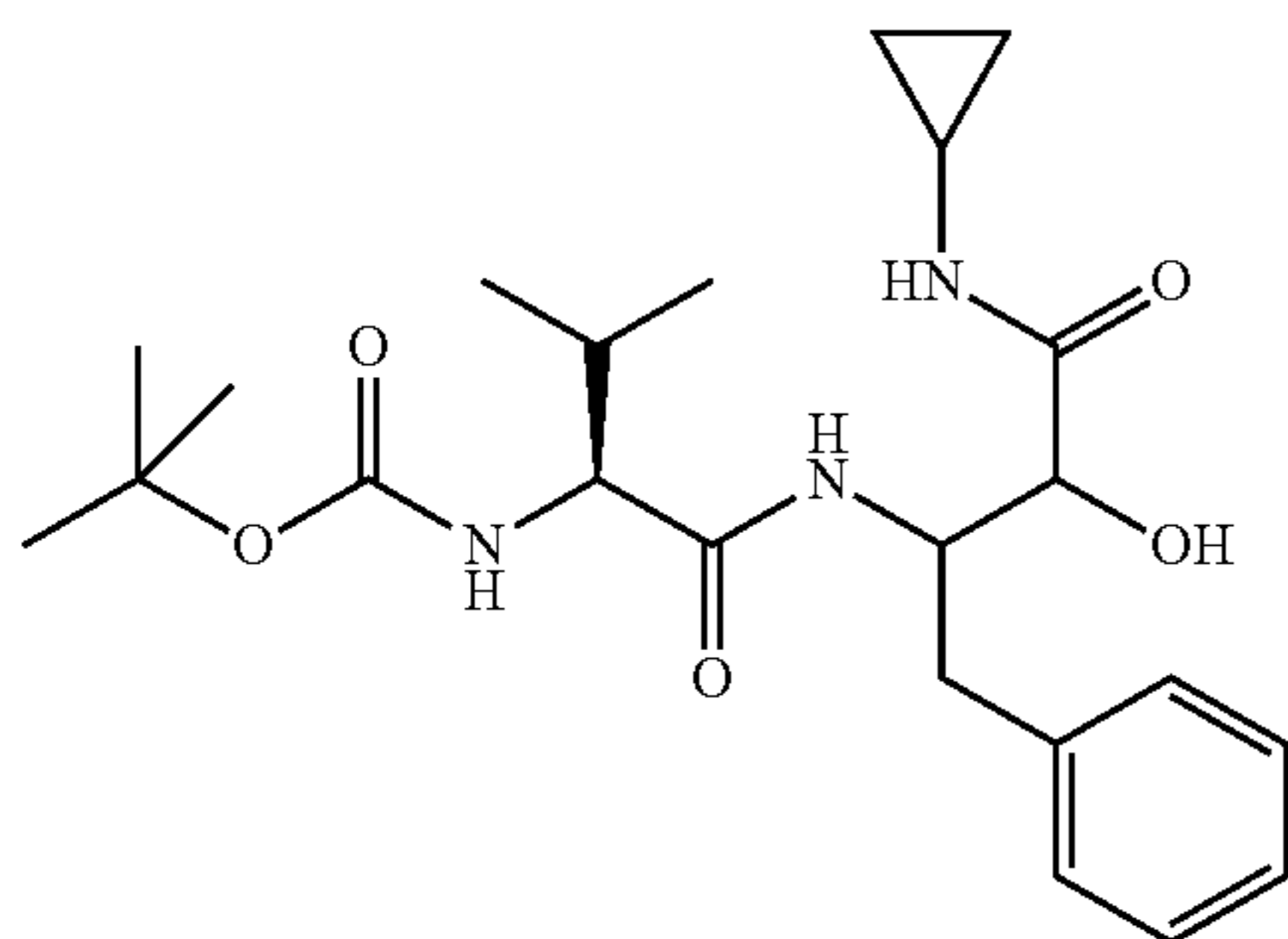
Intermediate 7: N-((2S)-1-(((2S)-4-(cyclopropylamino)-3-hydroxy-4-oxo-1-phenylbutan-2-yl)amino)-4-methyl-1-oxopentan-2-yl)-3-methoxybenzamide



A solution of intermediate 6 (115 mg, 0.3 mmol, 1.0 eq.) and DIPEA (231 μ l, 1.3 mmol, 4.0 eq.) in DCM (5 mL) was added to a solution of 3-methoxybenzoic acid (60.4 mg, 0.4 mmol, 1.2 eq.), EDC (108 mg, 0.6 mmol, 1.7 eq.) and HOBt (86 mg, 0.6 mmol, 1.5 eq.) in DCM (4 mL). The reaction mixture was stirred for 1 hour at room temperature. The volatiles were removed under vacuum and the crude mixture was extracted with EtOAc and washed with a sat. solution of NaHCO_3 (2 \times 20 mL). The material was purified by flash chromatography (ISCO Rf) using Hexane/Hexane:Ethanol (8:2) as eluents, from 0% to 60% in Hexane:Ethanol (8:2), product eluted at 40%. 100 mg product was obtained (0.2 mmol, 100%).

LC-MS: t_R =2.47 min; m/z =482

Intermediate 8: Synthesis of tert-butyl ((2S)-1-((4-(cyclopropylamino)-3-hydroxy-4-oxo-1-phenylbutan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate



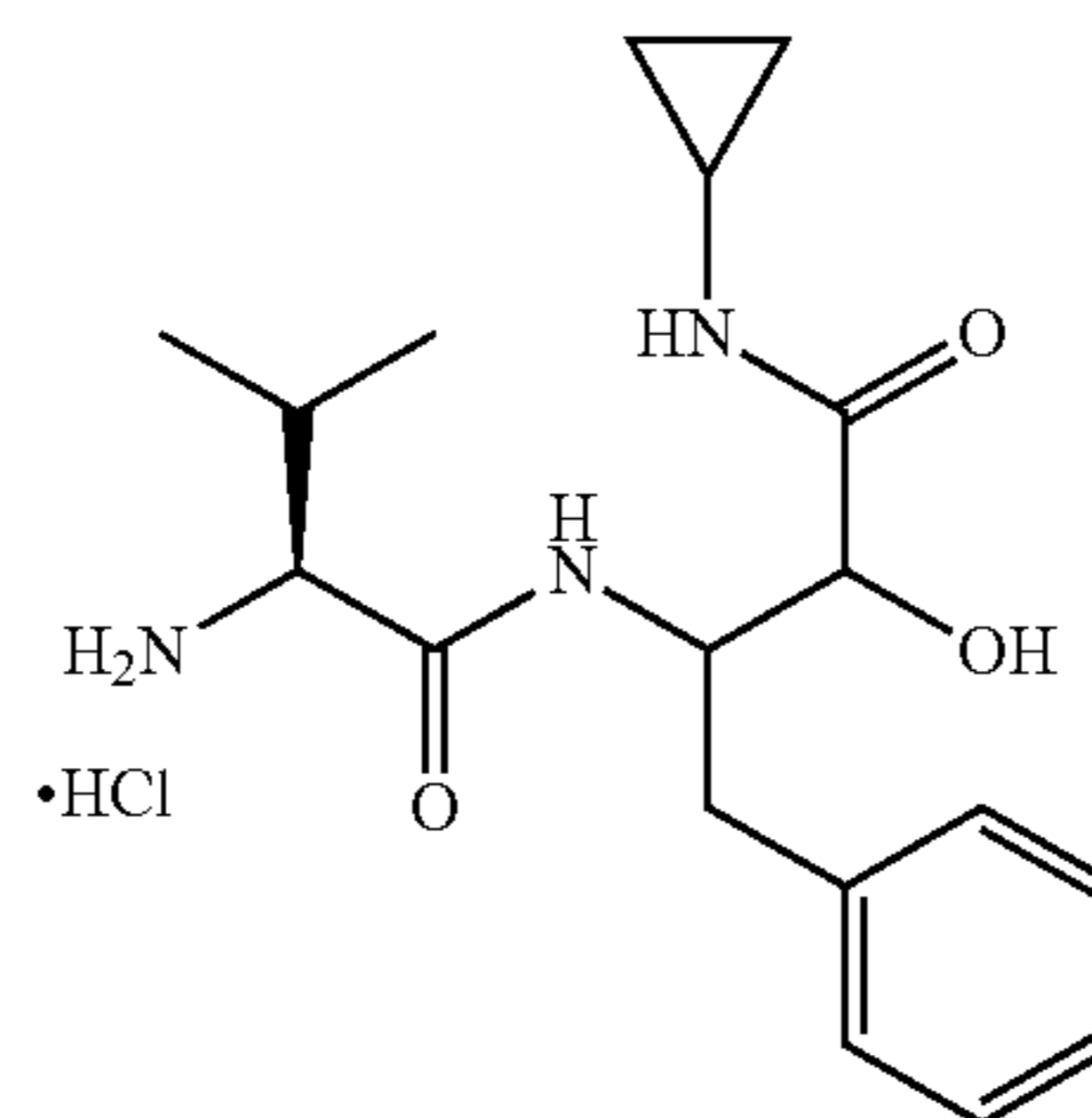
A solution of intermediate 4 (1.06 g, 3.91 mmol, 1.0 eq.) and DIPEA (2.74 mL, 15.66 mmol, 4 eq.) in DCM (5 mL) was added to a solution of Boc-L-Val-OH (1.28 g, 5.87 mmol, 1.5 eq.), EDC (1.13 g, 5.87 mmol, 1.5 eq.) and HOBt (0.9 g, 5.87 mmol, 1.5 eq.) in DCM (5 mL). The reaction mixture was stirred for 45 minutes at room temperature and then the volatiles were removed under vacuum. The crude mixture was extracted with EtOAc and washed with a sat. solution of NaHCO_3 . The crude material was purified by flash chromatography (ISCO Rf) using DCM/DCM:Methanol (8:2) as eluents, from 0% to 50% in DCM:Methanol

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(8:2), product eluted at 15%. 600 mg of product was obtained (1.384 mmol, 35% yield).

LC-MS: t_R =2.47 min; m/z =434

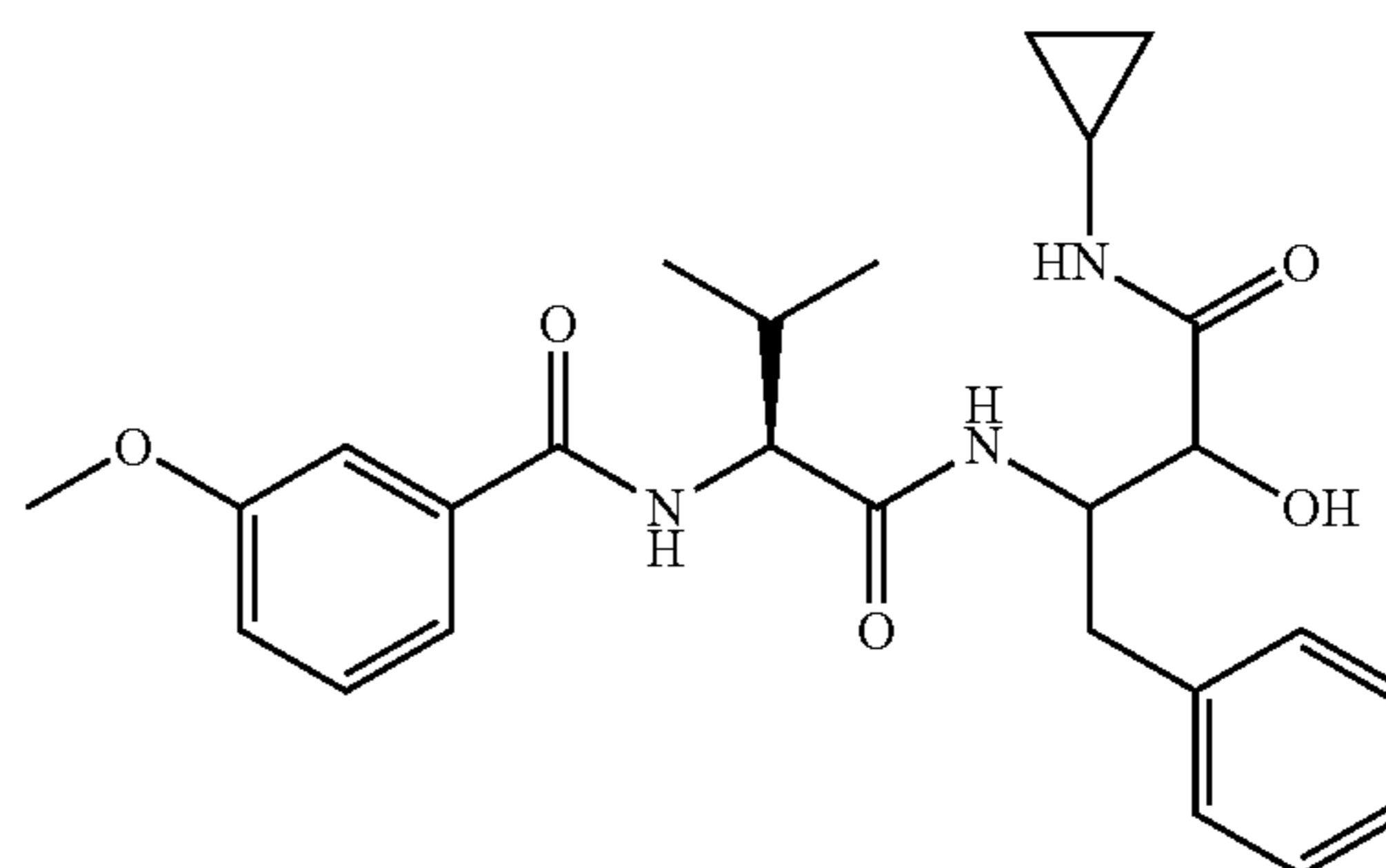
Intermediate 9: Synthesis of 3-((S)-2-amino-3-methylbutanamido)-N-cyclopropyl-2-hydroxy-4-phenylbutanamide hydrochloride



A solution of HCl 4 N in 1,4-dioxane (6.9 mL, 27.7 mmol, 20 eq.) was added to intermediate 8 (600 mg, 1.384 mmol, 1.0 eq.). The mixture was stirred at room temperature for 1 h and then evaporated to dryness. The product was used for next step without further purification.

LC-MS: t_R =1.77-1.88 min; m/z =334

Intermediate 10: Synthesis of N-((2S)-1-((4-(cyclopropylamino)-3-hydroxy-4-oxo-1-phenylbutan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-3-methoxybenzamide

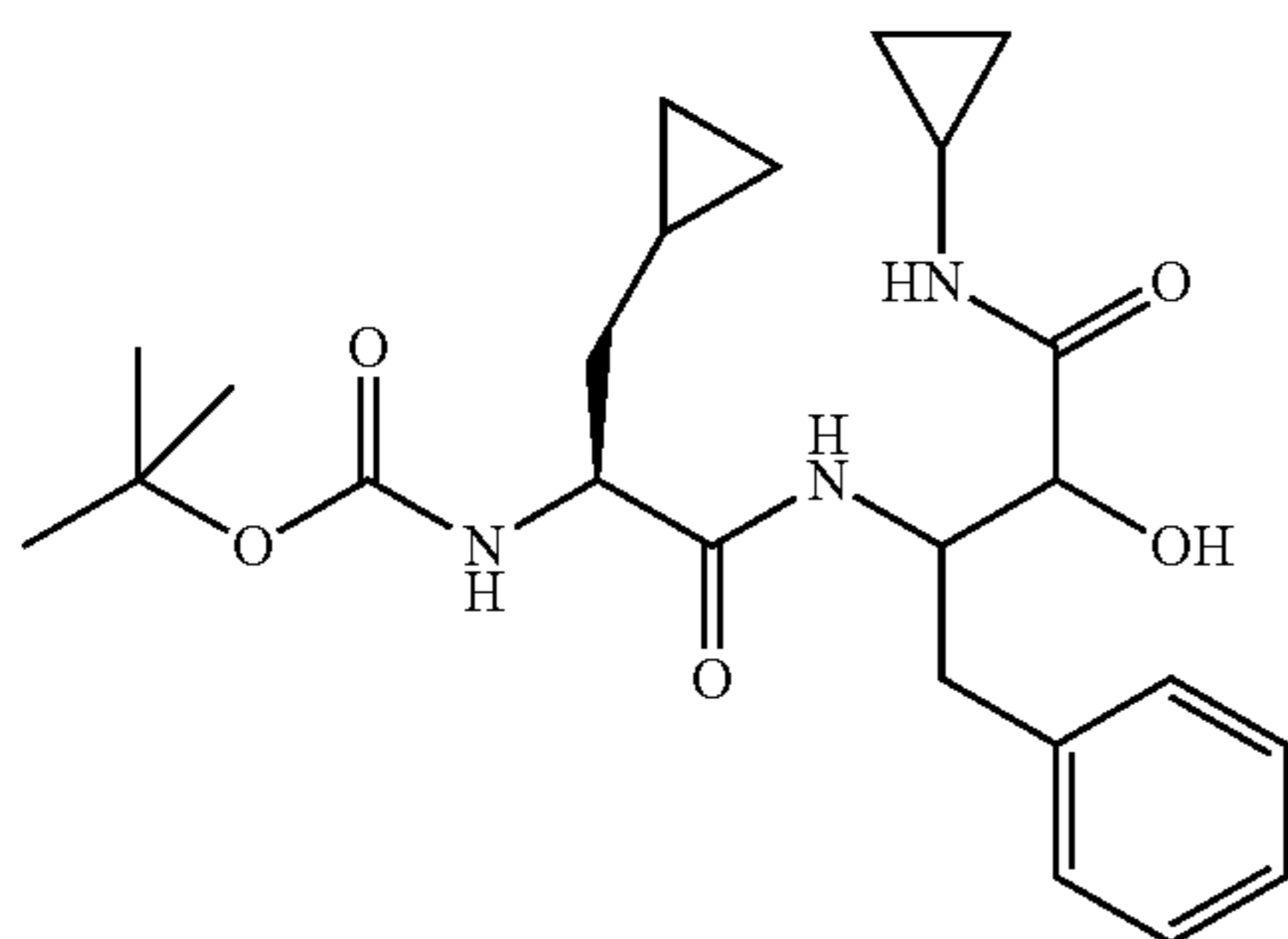


A solution of intermediate 9 (461 mg, 0.3 mmol, 1.0 eq.) and DIPEA (0.97 mL, 5.53 mmol, 4.0 eq.) in DCM (9 mL) was added to a solution of 3-methoxybenzoic acid (316 mg, 2.07 mmol, 1.5 eq.), EDC (398 mg, 2.07 mmol, 1.5 eq.) and HOBt (318 mg, 2.07 mmol, 1.5 eq.) in DCM (9 mL). The reaction mixture was stirred for 1 hour at room temperature and then the volatiles were removed under vacuum. The crude mixture was extracted with EtOAc and washed with a sat. solution of NaHCO_3 . The crude material was purified by flash chromatography (ISCO Rf) using DCM/DCM:Methanol (8:2) as eluents, from 0% to 20% in DCM:Methanol (8:2), product eluted at 15%. 470 mg of product was obtained (1 mmol, 73%).

LC-MS: t_R =2.32 min; m/z =468

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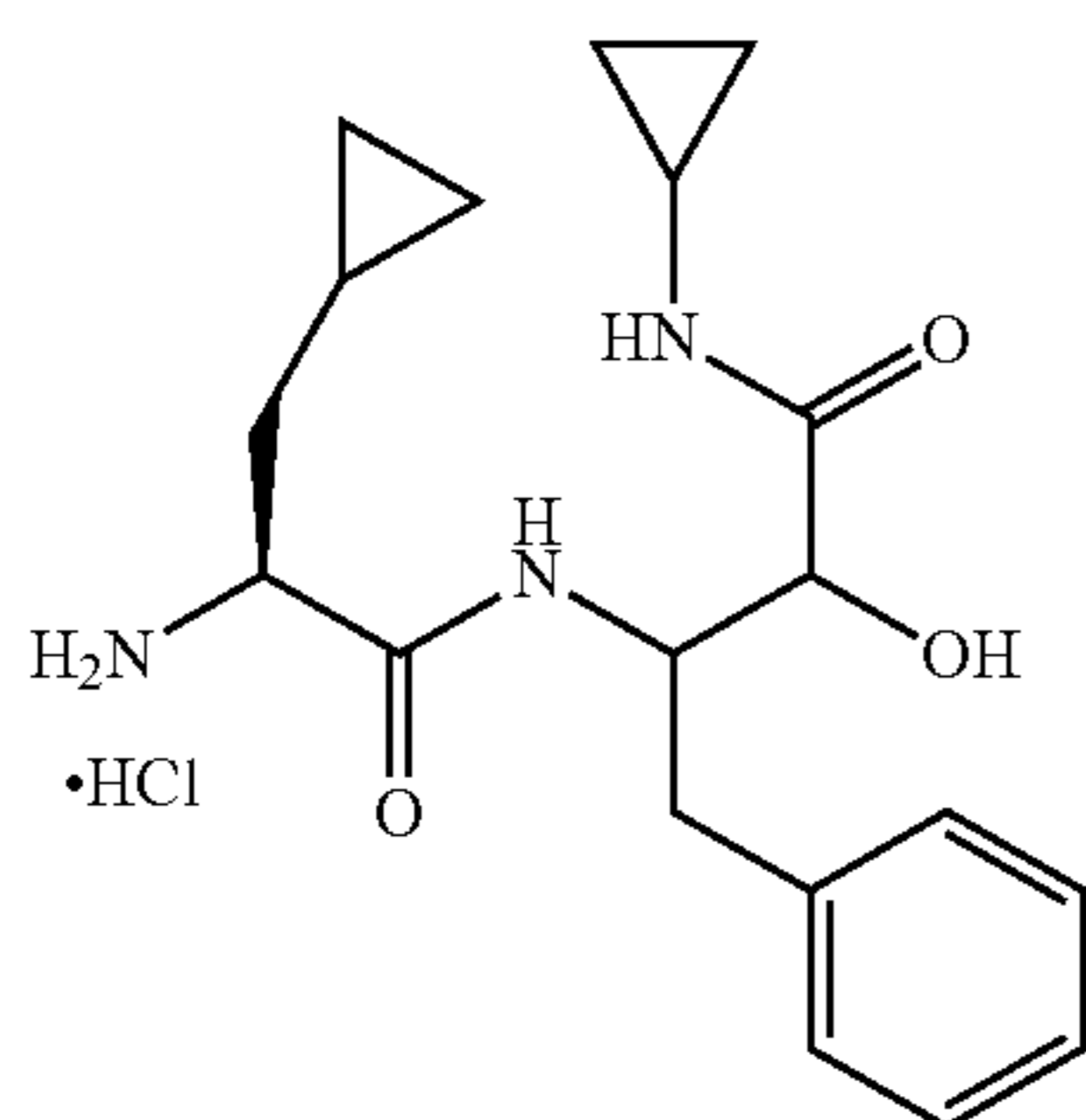
Intermediate 11: Synthesis of tert-butyl ((2S)-3-cyclopropyl-1-((4-(cyclopropylamino)-3-hydroxy-4-oxo-1-phenylbutan-2-yl)amino)-1-oxopropan-2-yl) carbamate



A solution of intermediate 4 (530 mg, 1.96 mmol, 1.0 eq.) and DIPEA (1.37 ml, 7.83 mmol, 4 eq.) in DCM (3 mL) was added to a solution of (S)-2-(Boc-amino)-3-cyclopropylpropanoic acid (673 mg, 2.94 mmol, 1.5 eq.), EDC (563 mg, 2.94 mmol, 1.5 eq.) and HOBt (450 mg, 2.94 mmol, 1.5 eq.) in DCM (2 mL). The reaction mixture was stirred for one and a half hours at room temperature. The volatiles were removed under vacuum and the crude mixture was extracted with EtOAc and washed with a sat. solution of NaHCO₃. The crude mixture was purified on flash chromatography (ISCO Rf) using DCM/DCM:Methanol (8:2) as eluents, from 0% to 20% in DCM:Methanol (8:2), product eluted at 12%. 430 mg of product was obtained (0.96 mmol, 49% yield).

LC-MS: $t_R=2.5$ min; $m/z=446$

Intermediate 12: Synthesis of 3-((S)-2-amino-3-cyclopropylpropanamido)-N-cyclopropyl-2-hydroxy-4-phenylbutanamide hydrochloride



A solution of HCl 4 N in 1,4-Dioxane (4.83 ml, 19.3 mmol, 20 eq.) was added to Intermediate 11 (430 mg, 0.96 mmol, 1.0 eq.). The mixture was stirred at room temperature for 1 h and then vaporated to dryness. The product was used for next step without further purification.

LC-MS (Method A): $t_R=1.82-1.92$ min; $m/z=346$

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Intermediate 13: Synthesis of N-((2S)-3-cyclopropyl-1-((4-(cyclopropylamino)-3-hydroxy-4-oxo-1-phenylbutan-2-yl)amino)-1-oxopropan-2-yl)-3-methoxybenzamide

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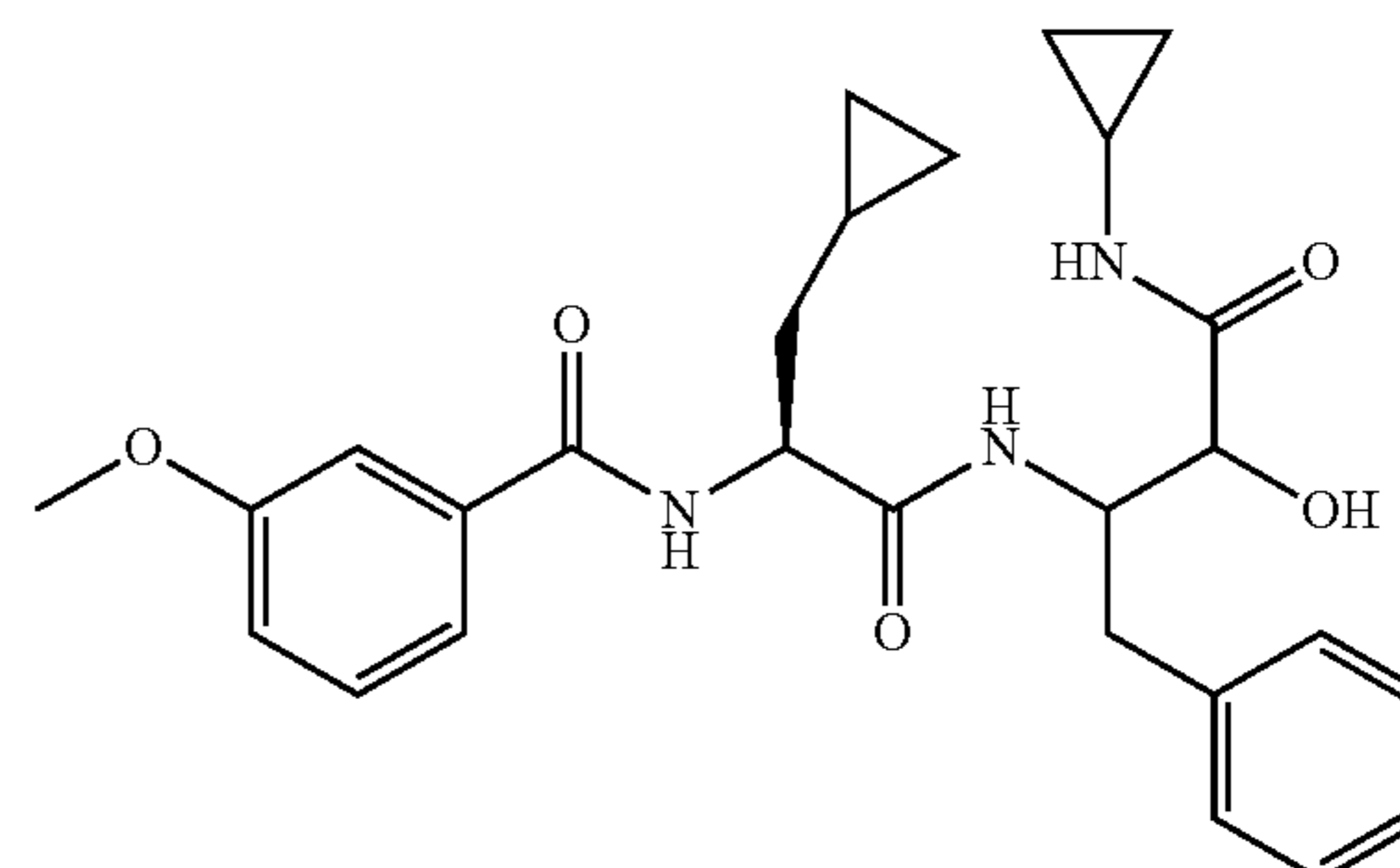
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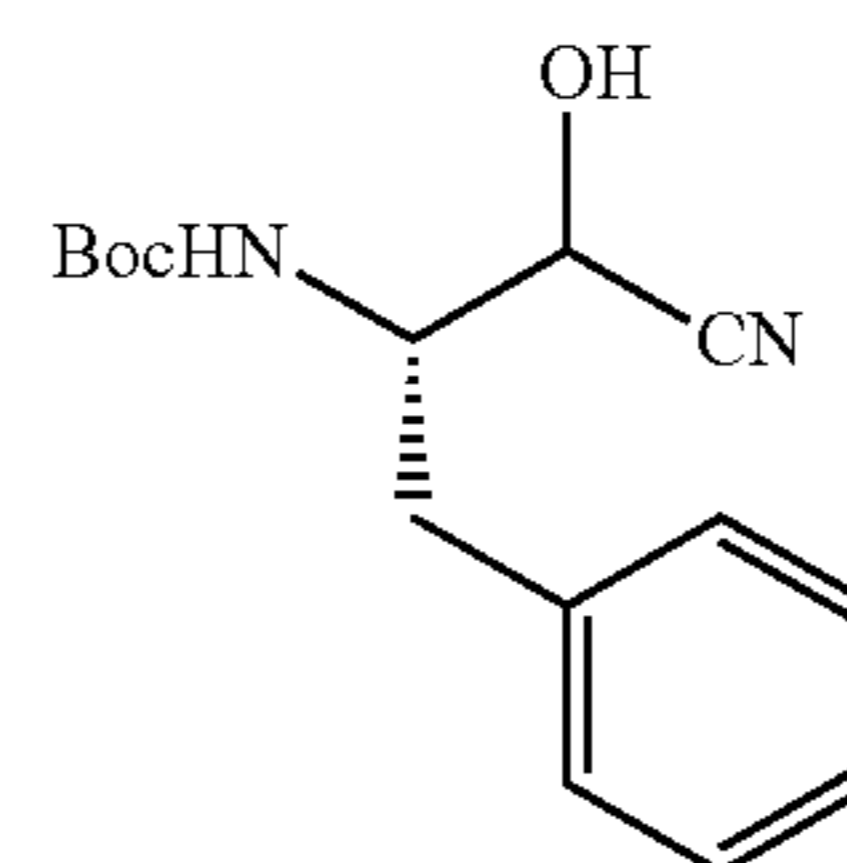
A solution of intermediate 12 (333 mg, 0.3 mmol, 1.0 eq.) and DIPEA (0.67 ml, 3.86 mmol, 4.0 eq.) in DCM (6 mL) was added to a solution of 3-methoxybenzoic acid (220 mg, 1.45 mmol, 1.5 eq.), EDC (277 mg, 1.45 mmol, 1.5 eq.) and HOBt (221 mg, 1.45 mmol, 1.5 eq.) in DCM (6 mL). The reaction mixture was stirred for 1 hour at room temperature and then the volatiles were removed under vacuum. The crude mixture was extracted with EtOAc and washed with a sat. solution of NaHCO₃. The crude material was purified on flash chromatography (ISCO Rf) using DCM/DCM:Methanol (8:2) as eluents, from 0% to 50% in DCM:Methanol (8:2), product eluted at 38%. 440 mg of product was obtained (0.88 mmol, 91%).

LC-MS: $t_R=2.37$ min; $m/z=480$

Intermediate 14: Synthesis of tert-butyl ((2S)-1-cyano-1-hydroxy-3-phenylpropan-2-yl)carbamate

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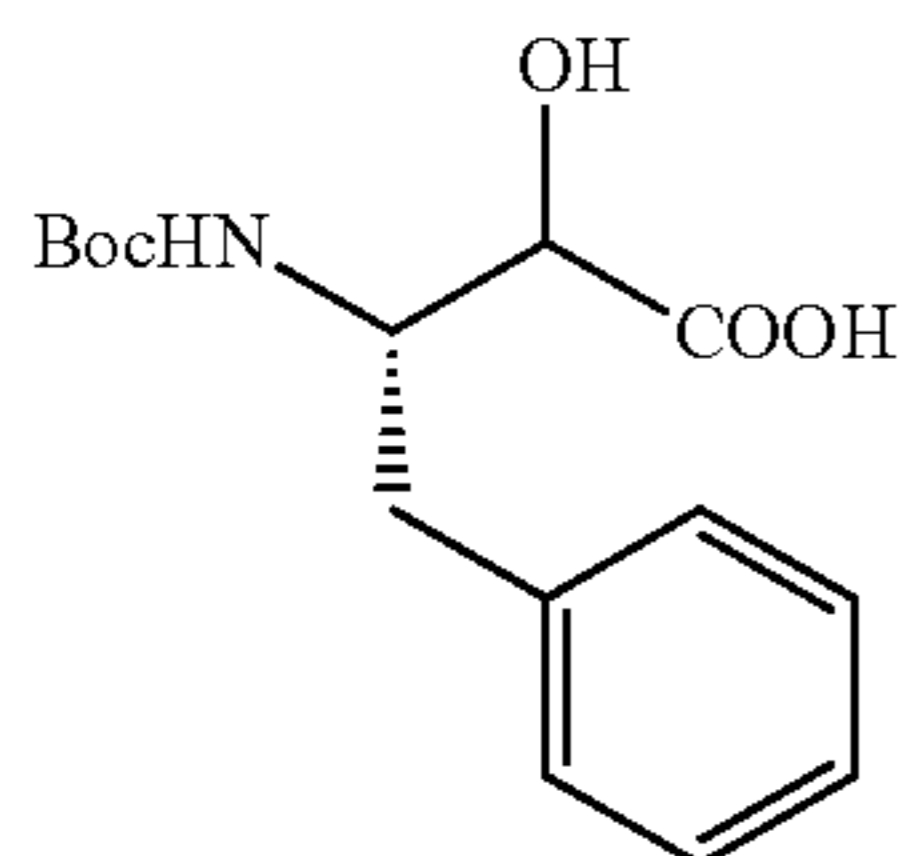


Sodium bisulfate (11.2 g, 107.0 mmol, 4 eq) was added to a solution of intermediate 2 (6.7 g, 26.9 mmol, 1.0 eq.) in 1,4-dioxane (150 ml) at 0° C. The reaction mixture was stirred at 0° C. for 10 minutes and potassium cyanide (7.0 g, 107.0 mmol, 4 eq) was added dissolved in water (45 mL). The reaction mixture was stirred overnight at room temperature. The organic solvent was evaporated, water and EtOAc were added and the layers separated. The combined organic layers were washed with sat. aq. NaHCO₃, dried and concentrated under reduced pressure. 7.0 g of product was obtained (23.5 mmol, 94% yield).

LC-MS: $t_R=2.83$ min; $m/z=277$

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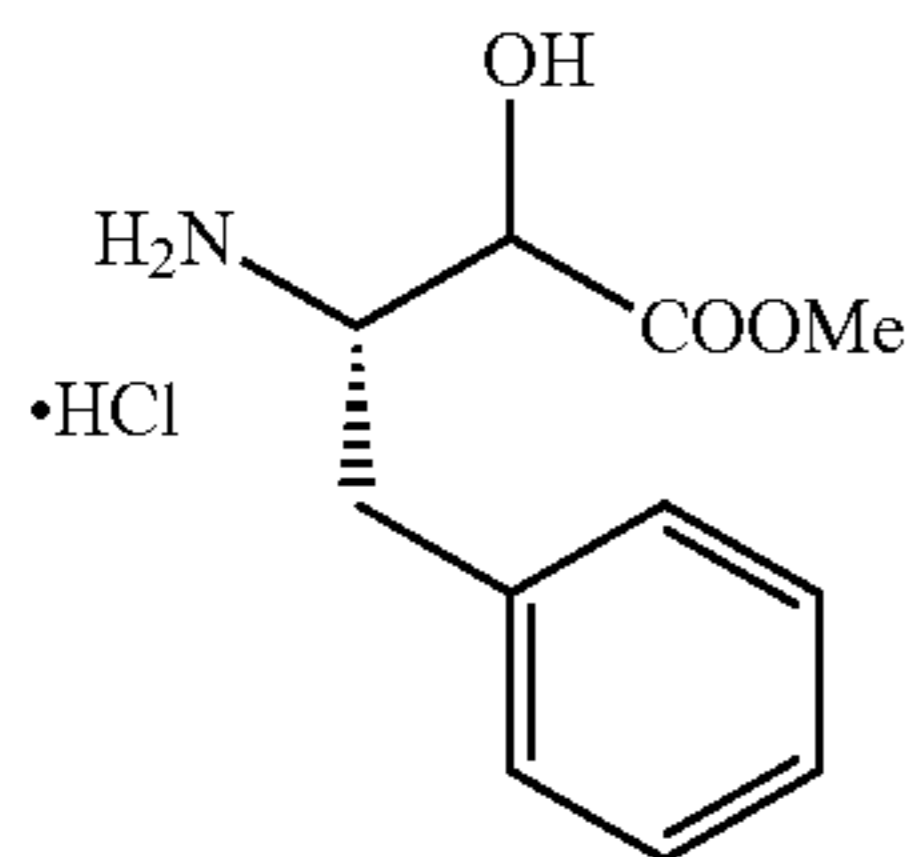
Intermediate 15: Synthesis of (3S)-3-((tert-butoxycarbonyl)amino)-2-hydroxy-4-phenylbutanoic acid



Intermediate 14 (6.5 g, 23.5 mmol, 1.0 eq.) was dissolved in 20 ml of conc. HCl solution and refluxed for 1 hour. Then, the crude mixture was cooled and washed with DCM. NaOH 10 N was added to the aqueous layer until pH 11, and washed with DCM. Boc₂O (5.6 g, 25.8 mmol, 1.1 eq) was added to the aqueous layer. The mixture was stirred at room temperature overnight. After total conversion was achieved, the mixture was acidified with KHSO₄ to pH 2 and extracted with EtOAc. The organic layer was evaporated and the product was used for next step without further purification. 5.1 g was obtained (17.2 mmol, 73% yield 2 steps).

LC-MS: $t_R=1.62$ min; $m/z=296$

Intermediate 16: Synthesis of (3S)-methyl 3-amino-2-hydroxy-4-phenylbutanoate hydrochloride



Oxalyl chloride (6 ml, 69.9 mmol, 4.0 eq.) was added slowly to a solution of intermediate 15 (5.1 g, 17.26 mmol, 1.0 eq.) in MeOH (300 ml) at 0° C. The mixture was stirred overnight at room temperature. The crude mixture was evaporated and then coevaporated with MeOH and the product was used for next step without further purification. 4.2 g was obtained (17.26 mmol, Quantitative yield).

LC-MS: $t_R=1.58-1.65$ min; $m/z=210$

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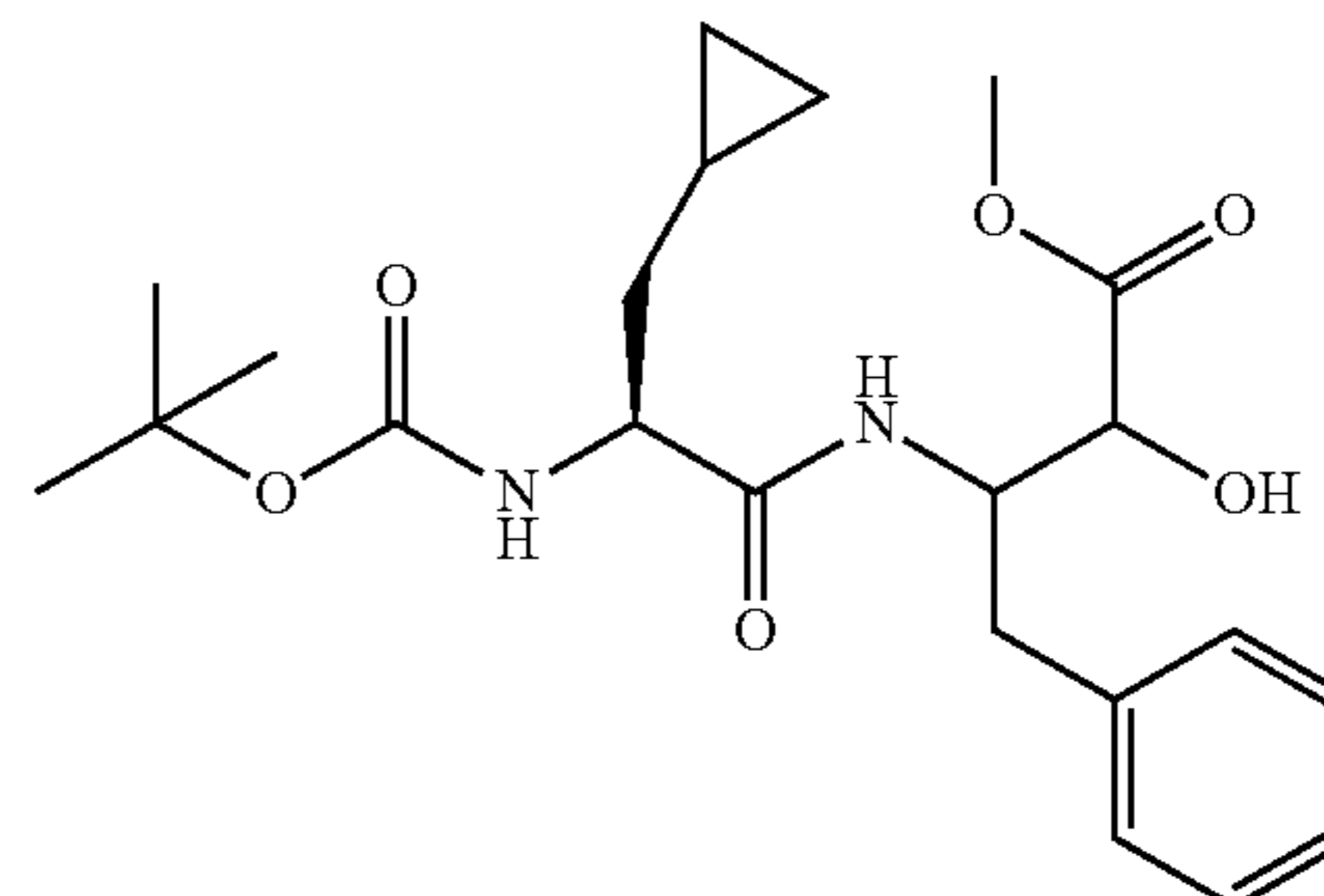
Intermediate 17: Synthesis of methyl 3-((S)-2-((tert-butoxycarbonyl)amino)-3-cyclopropylpropanamido)-2-hydroxy-4-phenylbutanoate

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A solution of intermediate 16 (3 g, 12.21 mmol, 1.0 eq.) and DIPEA (8.53 ml, 48.8 mmol, 4 eq.) in DCM (15 mL) was added to a solution of (S)-2-(Boc-amino)-3-cyclopropylpropanoic acid (2.8 g, 12.21 mmol, 1 eq.), EDC (3.51 g, 18.31 mmol, 1.5 eq.) and HOBt (2.8 g, 18.31 mmol, 1.5 eq.) in DCM (10 mL). The reaction mixture was stirred for 1 hour at room temperature. The volatiles were removed under vacuum. The crude mixture was extracted with EtOAc and washed with a sat. solution of NaHCO₃. The crude product was purified on flash chromatography (Biotage) using DCM/DCM:Methanol (8:2) as eluents, from 0% to 30% in DCM:Methanol (8:2), product eluted at 20%. 2.2 g of product was obtained (5.23 mmol, 43% yield).

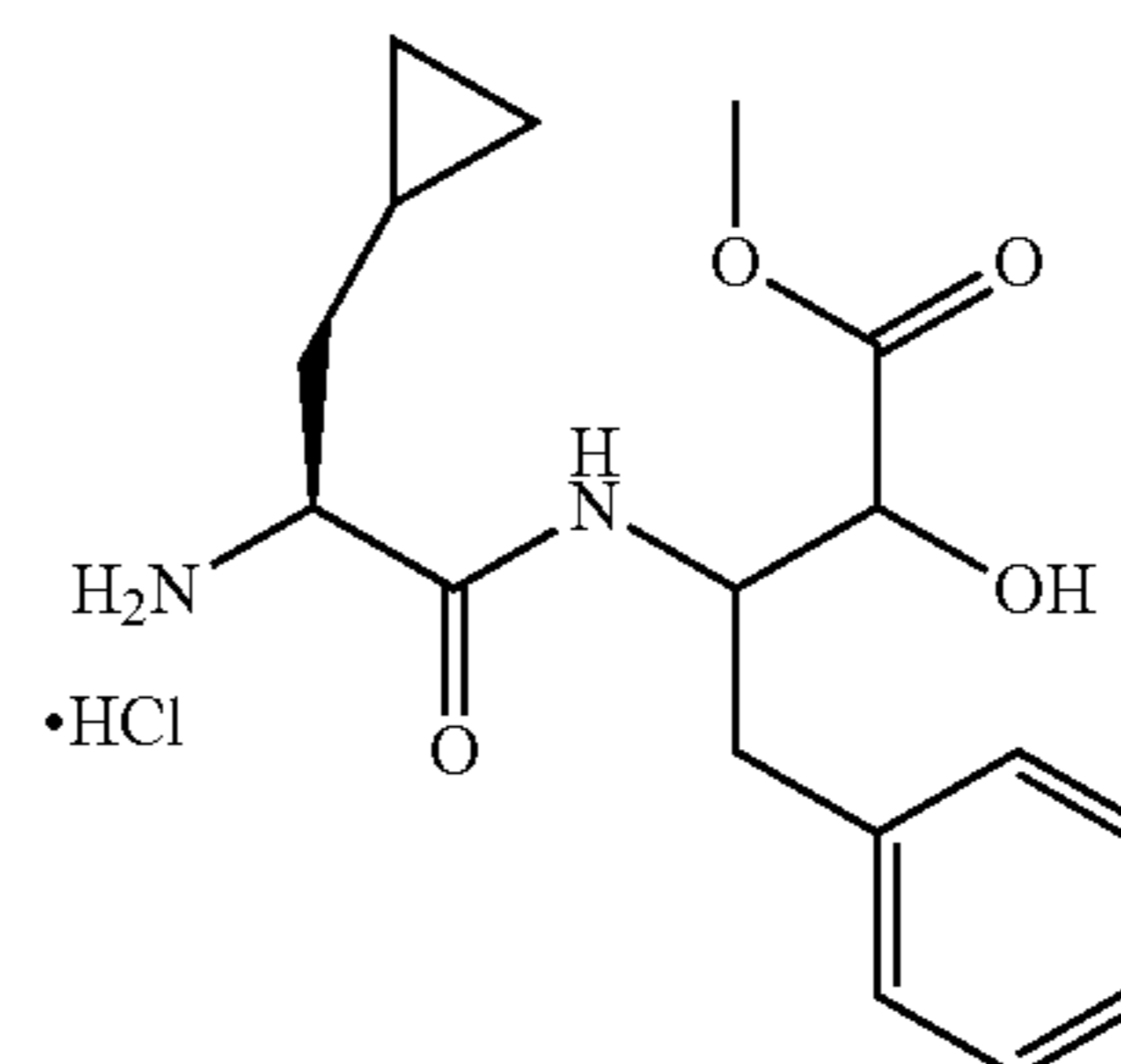
LC-MS: $t_R=2.64$ min; $m/z=421$

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Intermediate 18: Synthesis of methyl 3-((S)-2-amino-3-cyclopropylpropanamido)-2-hydroxy-4-phenylbutanoate hydrochloride

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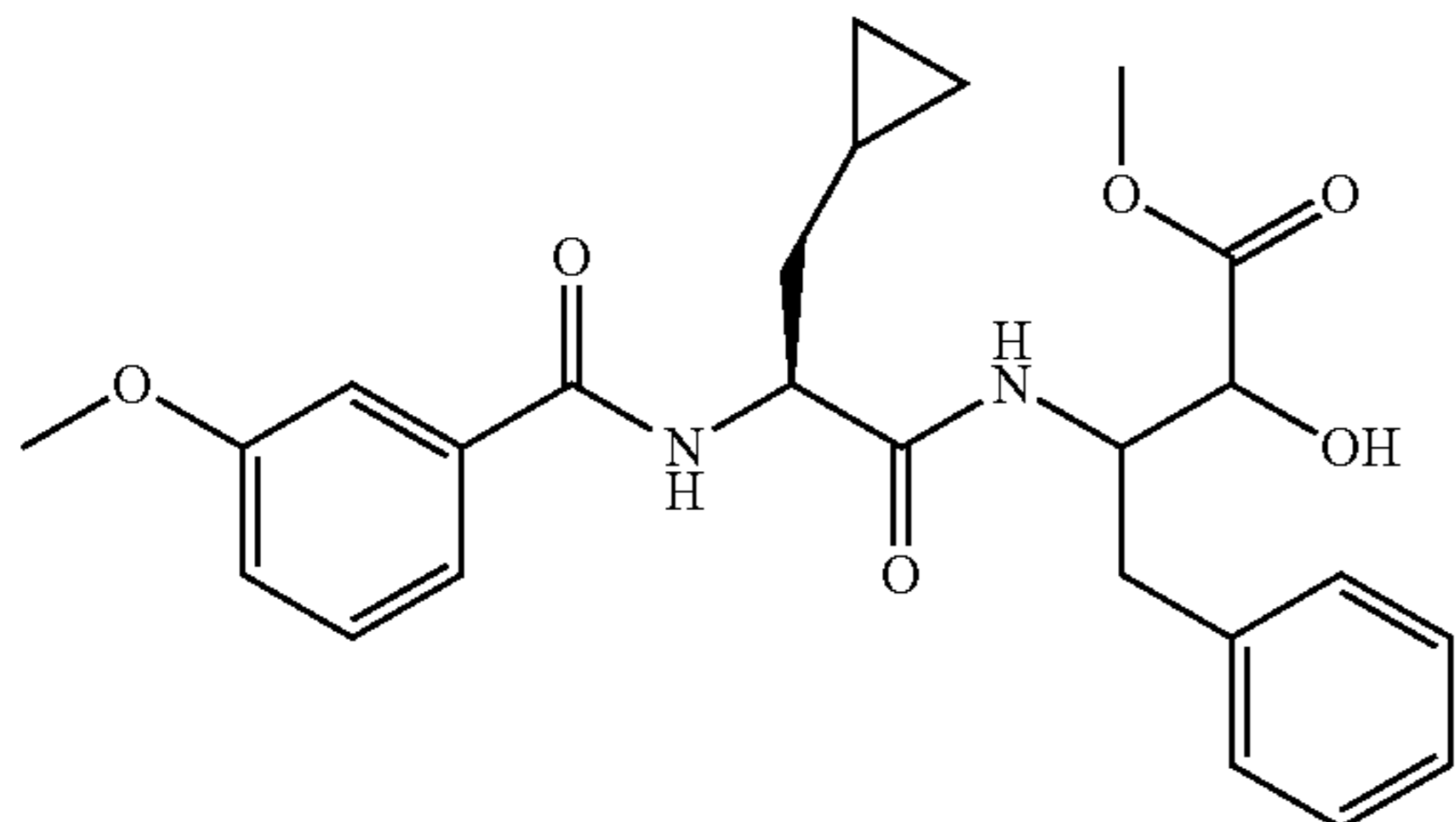
A solution of HCl 4 N in 1,4-dioxane (26.2 ml, 105 mmol, 20 eq.) was added to intermediate 17 (2.2 g, 5.23 mmol, 1.0 eq.). The mixture was stirred at room temperature for 1 h and then evaporated to dryness. The product was used for next step without further purification.

LC-MS: $t_R=1.78$ min; $m/z=321$

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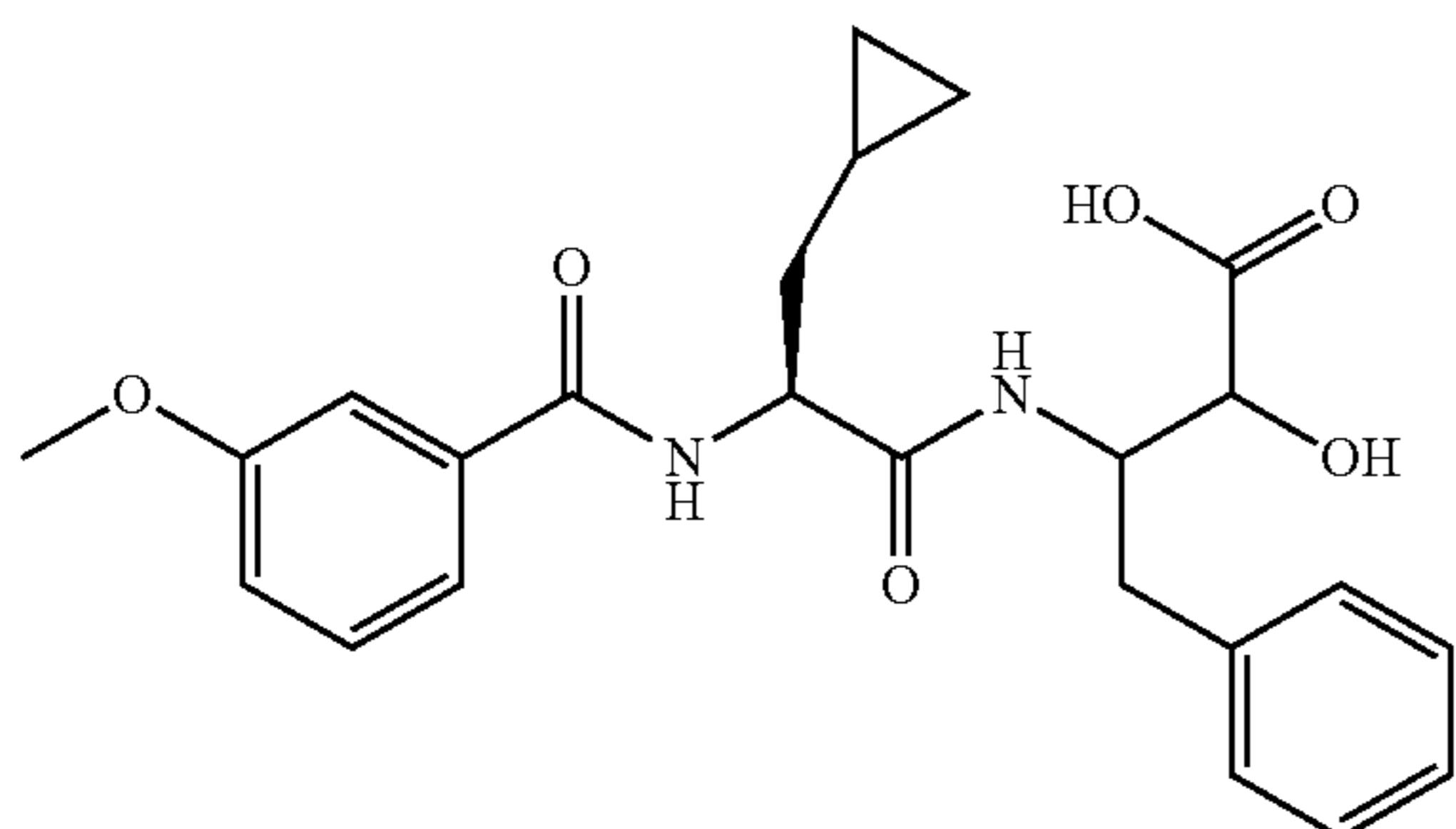
Intermediate 19: Synthesis of methyl 3-((S)-3-cyclopropyl-2-(3-methoxybenzamido)propanamido)-2-hydroxy-4-phenylbutanoate



A solution of intermediate 18 (1.87 g, 5.23 mmol, 1.0 eq.) and DIPEA (3.66 ml, 3.86 mmol, 4.0 eq.) in DCM (10 mL) was added to a solution of 3-methoxybenzoic acid (0.955 g, 6.28 mmol, 1.2 eq.), EDC (1.5 g, 7.85 mmol, 1.5 eq.) and HOBt (1.2 g, 7.85 mmol, 1.5 eq.) in DCM (10 mL). The reaction mixture was stirred for 1 hour at room temperature and then the volatiles were removed under vacuum. The crude mixture was extracted with EtOAc and washed with a sat. solution of NaHCO₃. The crude product was purified by flash chromatography (Biotage) using DCM/DCM:Methanol (8:2) as eluents, from 0% to 50% in DCM:Methanol (8:2), product eluted at 25%. 1 g of product was obtained (2.2 mmol, 42%).

LC-MS: t_R =2.47 min; m/z =455

Intermediate 20: Synthesis of 3-((S)-3-cyclopropyl-2-(3-methoxybenzamido)propanamido)-2-hydroxy-4-phenylbutanoic acid



LiOH.H₂O (111 mg, 2.64 mmol, 3.0 eq.) was added to a solution of intermediate 19 (400 mg, 0.88 mmol, 1.0 eq.) dissolved in 13.5 ml of a mixture THF/MeOH/H₂O (5/3/1). After total conversion was achieved, solvents were removed under vacuum. The crude material was dissolved in 1 M HCl and extracted with DCM until all Intermediate 20 had been extracted to the organic layer. The organic layers were evaporated to dryness. Intermediate 20 was used for next step without further purification.

LC-MS: t_R =1.85 min; m/z =441

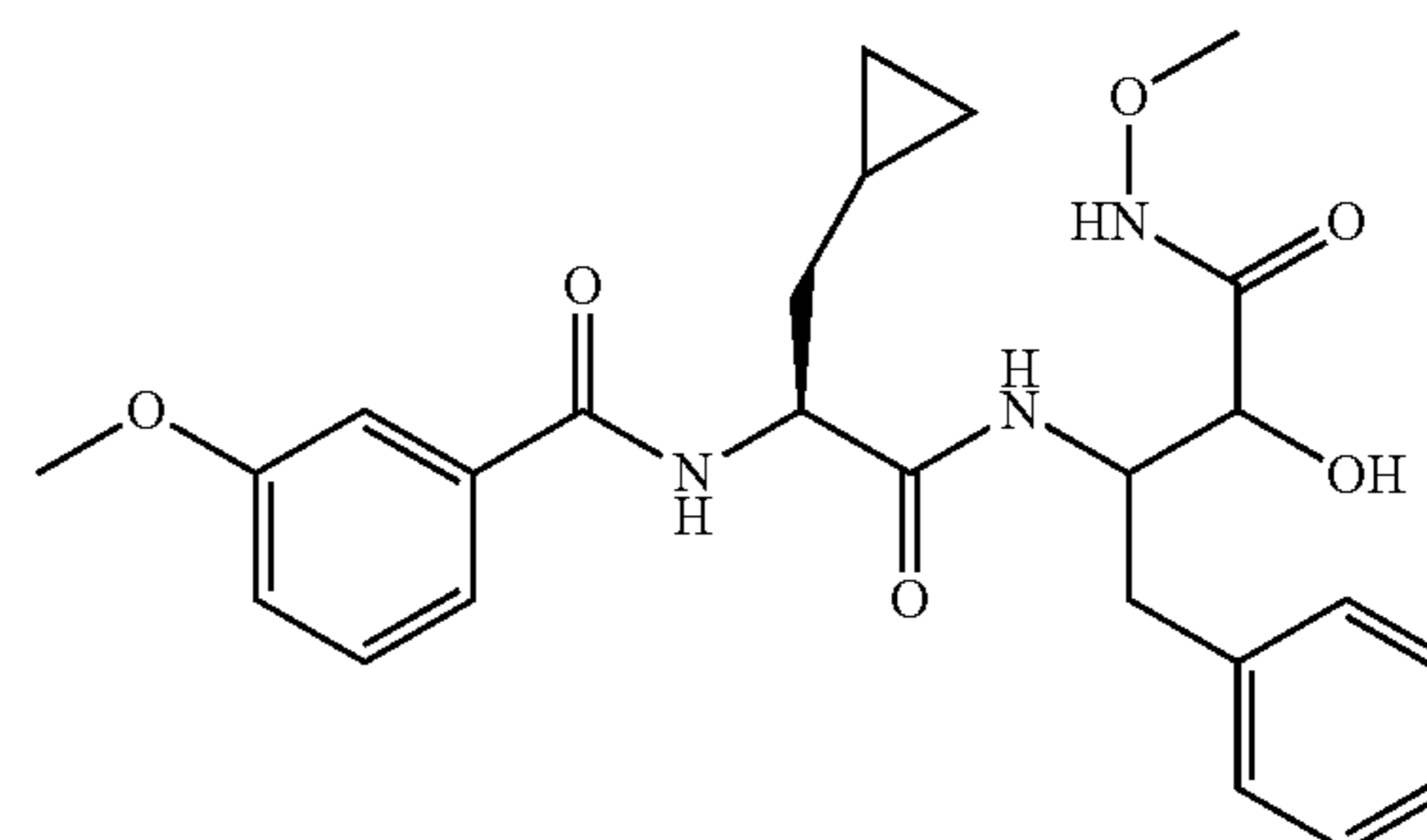
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Intermediate 21: Synthesis of N-((2S)-3-cyclopropyl-1-((3-hydroxy-4-(methoxyamino)-4-oxo-1-phenylbutan-2-yl)amino)-1-oxopropan-2-yl)-3-methoxybenzamide

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To a solution of Intermediate 20 (470 mg, 1.07 mmol, 1.0 eq.) and O-methylhydroxylamine hydrochloride (134 mg, 1.6 mmol, 1.5 eq.) in DCM (20 mL) was added EDC (307 mg, 1.6 mmol, 1.5 eq.) and HOBt (245 mg, 1.6 mmol, 1.5 eq.) and DIPEA (0.75 ml, 4.27 mmol, 4.0 eq.). The reaction mixture was stirred for 1 hour at room temperature. After this time 0.1 ml of DIPEA were added and the reaction stirred overnight at room temperature. 0.5 eq of both EDC and HOBt were added and stirring continued for 2 hours at room temperature. The volatiles were removed under vacuum. The crude mixture was extracted with EtOAc and washed with a sat. solution of NaHCO₃ and an aqueous solution of citric acid 5%. The crude product was purified on flash chromatography (Biotage) using DCM/DCM:Methanol (8:2) as eluents, from 0% to 50% in DCM:Methanol (8:2), product eluted at 20%. 170 mg of product was obtained. (0.37 mmol, 35% yield).

LC-MS: t_R =2.18 min; m/z =470

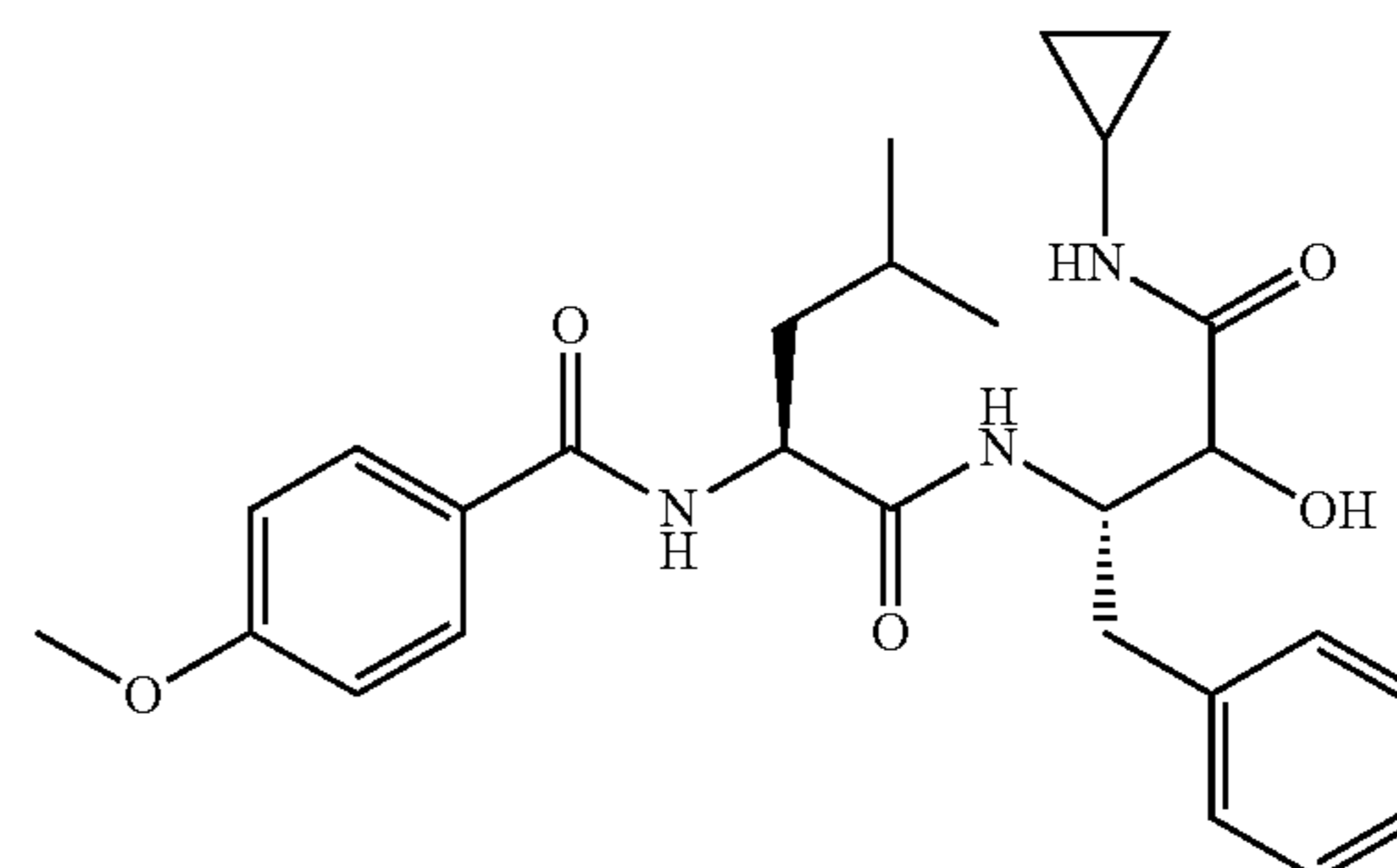
Intermediate 22: N-((2S)-1-(((2S)-4-(cyclopropylamino)-3-hydroxy-4-oxo-1-phenylbutan-2-yl)amino)-4-methyl-1-oxopentan-2-yl)-4-methoxybenzamide

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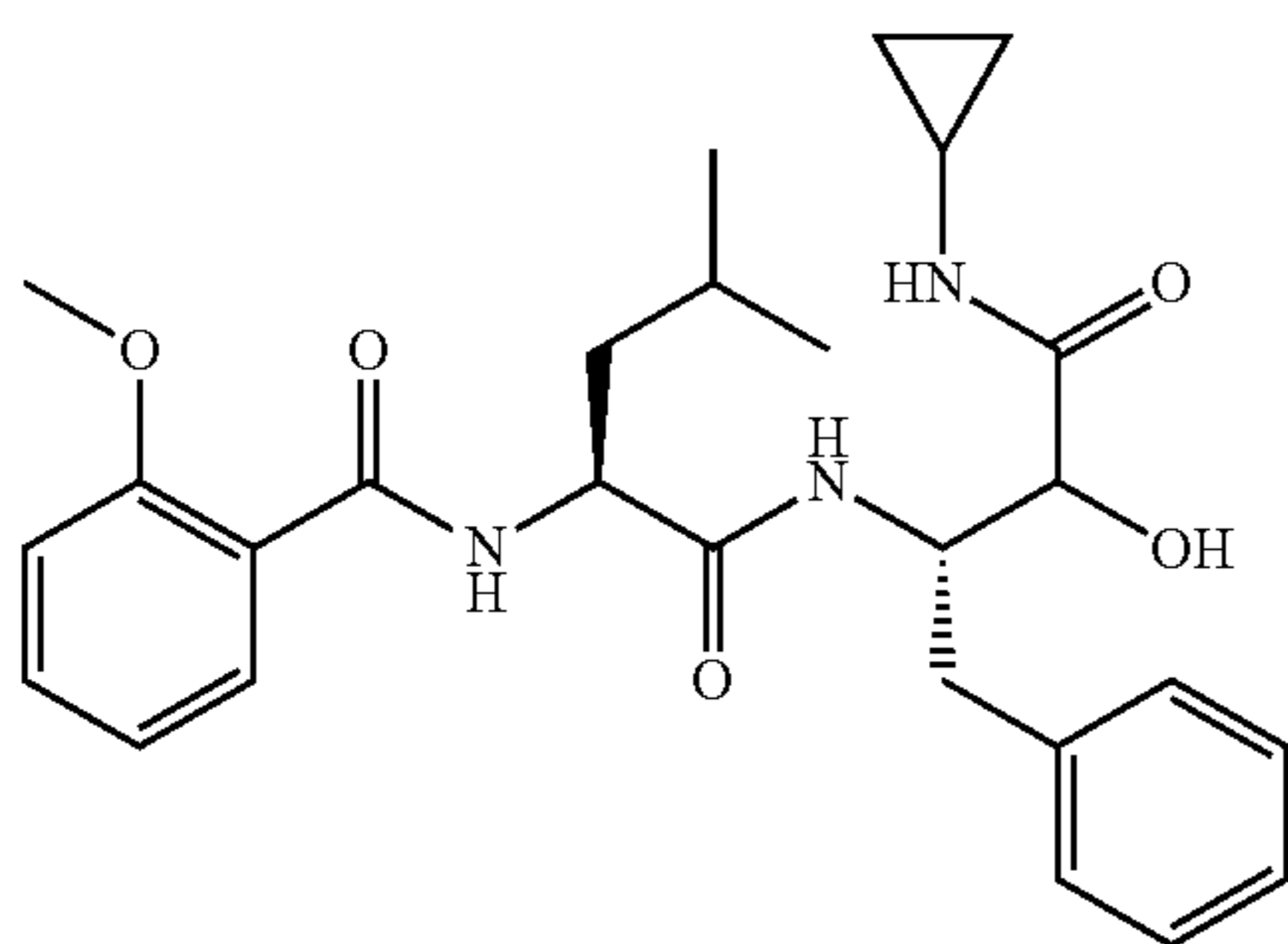
A solution of intermediate 6 (200 mg, 0.6 mmol, 1.0 eq.) and DIPEA (392 μ l, 2.3 mmol, 4.0 eq.) in DCM (6 mL) was added to a solution of 4-methoxybenzoic acid (105 mg, 0.7 mmol, 1.2 eq.), EDC (188 mg, 1.0 mmol, 1.7 eq.) and HOBt (150 mg, 1.0 mmol, 1.7 eq.) in DCM (6 mL). The reaction mixture was stirred for 1 hour at room temperature and the volatiles were removed under vacuum. The crude was extracted with EtOAc and washed with a sat. solution of NaHCO₃ (3 \times 10 ml) and an aqueous solution of citric acid

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5% (3×10 ml). The crude mixture was purified by flash chromatography (ISCO Rf) using Hexane/Hexane:Ethanol (8:2) as eluents, from 0% to 100% in Hexane:Ethanol (8:2), product eluted at 35%. 115 mg of product was obtained (0.2 mmol, 410%).

LC-MS: $t_R=2.35$ min; $m/z=482$

Intermediate 23: N-((2S)-1-(((2S)-4-(cyclopropylamino)-3-hydroxy-4-oxo-1-phenylbutan-2-yl)amino)-4-methyl-1-oxopentan-2-yl)-2-methoxybenzamide

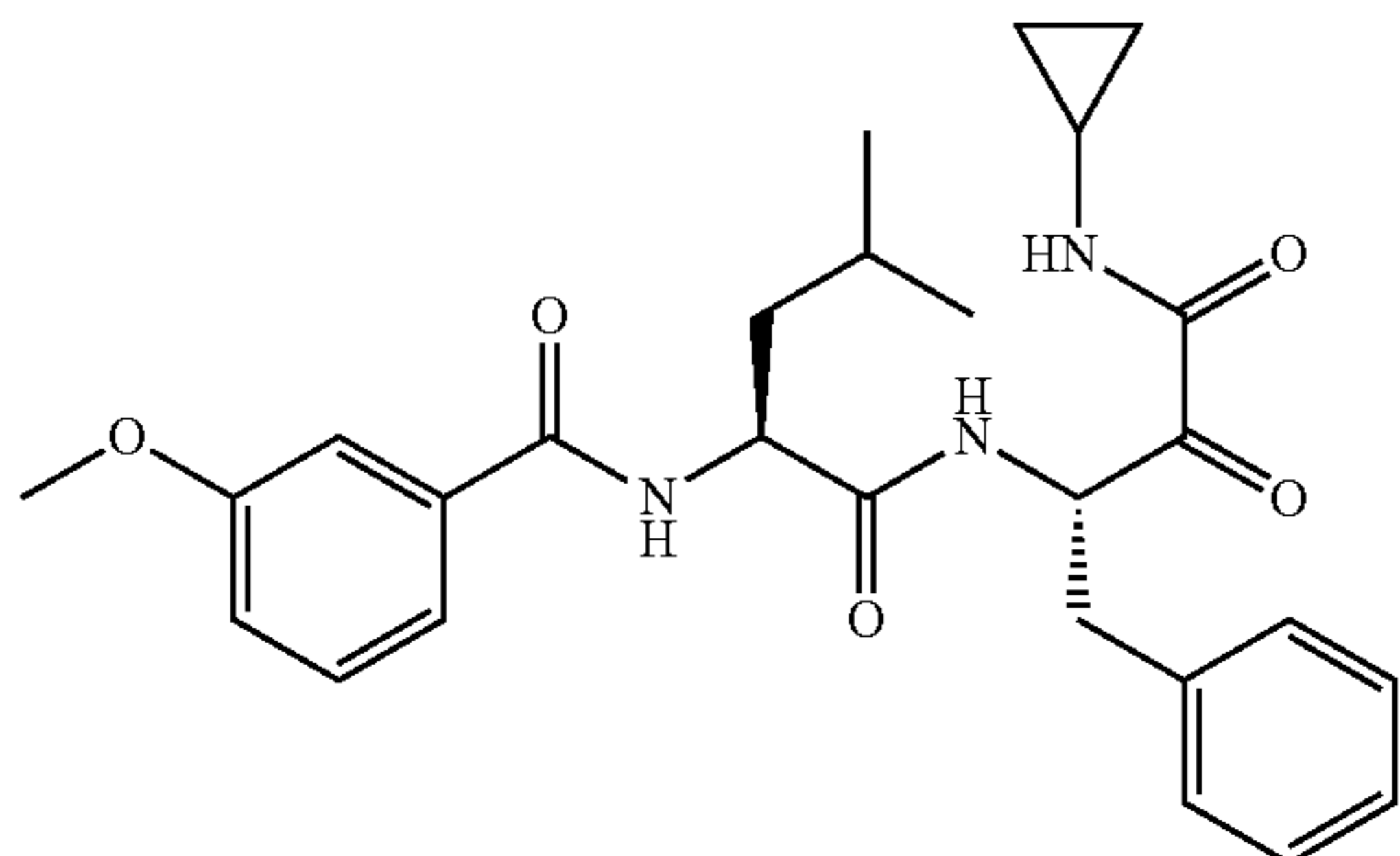


A solution of intermediate 6 (200 mg, 0.5 mmol, 1.0 eq.) and DIPEA (350 μ l, 2.0 mmol, 4.0 eq.) in DCM (6 mL) was added to a solution of 2-methoxybenzoic acid (95 mg, 0.6 mmol, 1.2 eq.), EDC (169 mg, 0.9 mmol, 1.7 eq.) and HOBt (135 mg, 0.9 mmol, 1.7 eq.) in DCM (5 mL). The reaction mixture was stirred for 1 hour at room temperature and then the volatiles were removed under vacuum. The crude mixture was extracted with EtOAc and washed with a sat. solution of NaHCO_3 (3×10 ml) and an aqueous solution of citric acid 5% (3×10 ml). The crude product was purified on flash chromatography (ISCO Rf) using Hexane/Ethanol (8:2) as eluents, from 0% to 100% in Hexane:Ethanol (8:2), product eluted at 35%. 250 mg were obtained (0.5 mmol, 100%).

LC-MS: $t_R=2.47$ min; $m/z=482$.

PREPARATION OF EXAMPLES AND COMPARATIVE EXAMPLES

Example 1: N-((S)-1-(((S)-4-(cyclopropylamino)-3,4-dioxo-1-phenylbutan-2-yl)amino)-4-methyl-1-oxopentan-2-yl)-3-methoxybenzamide



5 μ l of water were added to a Dess-Martin Periodinane 15% solution in DCM (640 μ l, 0.3 mmol, 1.5 eq.). Then the

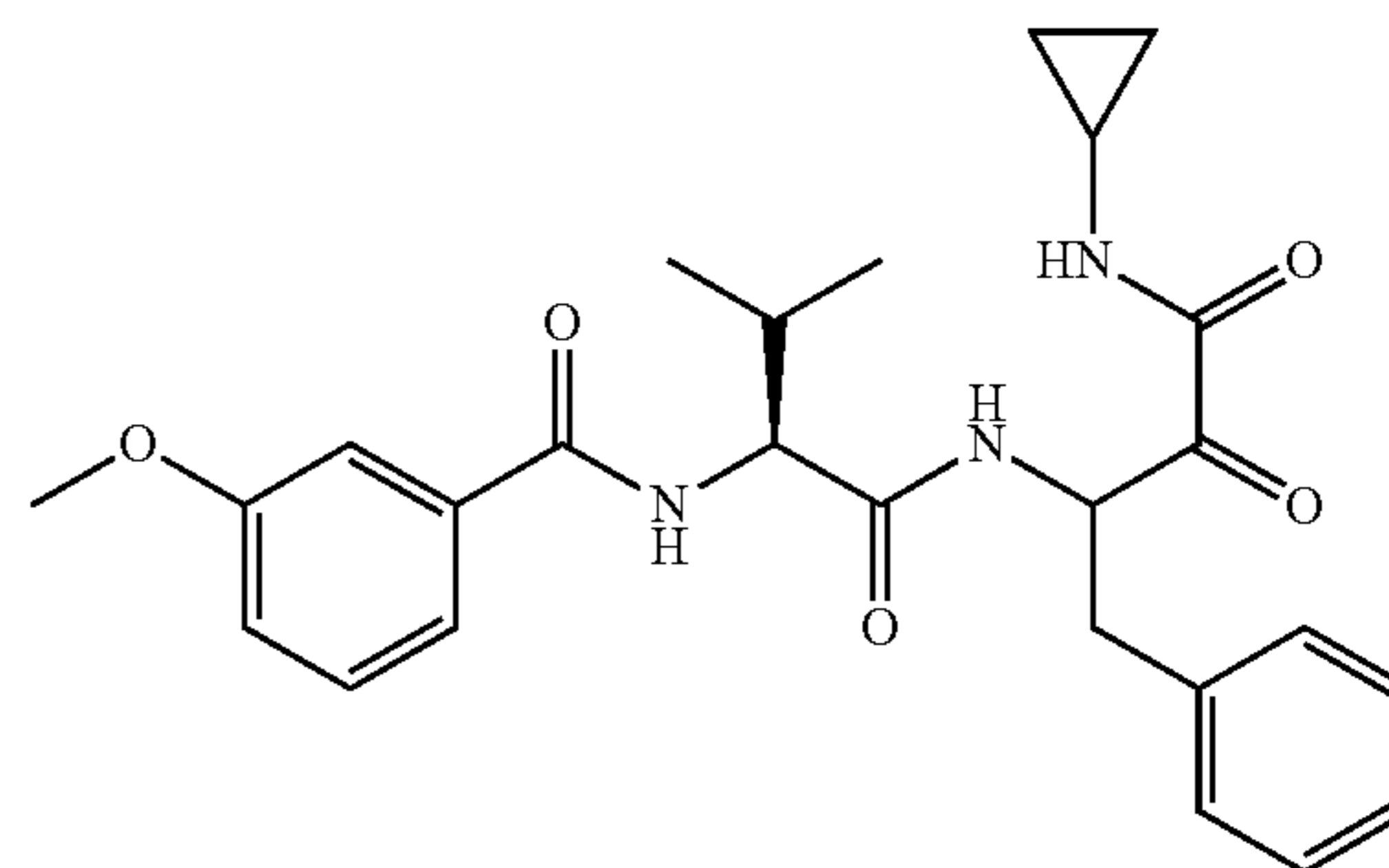
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mixture was added to a solution of intermediate 7 (100 mg, 0.2 mmol, 1.0 mmol) in DCM (4 ml). The mixture was stirred for 15 minutes at room temperature and then the volatiles were removed under vacuum. The crude mixture was extracted with EtOAc and washed with a sat. solution of NaHCO_3 (2×10 mL). The crude product was purified by flash chromatography (ISCO Rf) using Hexane/Ethanol (8:2) as solvents, from 0% to 60% in Hexane:Ethanol (8:2), product eluted at 30% in Hexane:Ethanol (8:2). 47.6 mg of product were obtained (0.1 mmol, 47% yield).

LC-MS: $t_R=2.63$ min; $m/z=480$

$^1\text{H NMR}$ (400 MHz, DMSO-d_6) δ 8.75 (dd, $J=26.3$, 5.1 Hz, 1H), 8.38 (dd, $J=18.9$, 8.1 Hz, 1H), 8.33 (d, $J=7.7$ Hz, 1H), 7.49-7.31 (m, 3H), 7.27-7.15 (m, 5H), 7.09 (m, 1H), 5.22-5.13 (m, 1H), 4.54 (m, 1H), 3.80 (s, 3H), 3.13 (m, 1H), 2.88-2.77 (m, 1H), 2.76-2.70 (m, 1H), 1.67-1.55 (m, 1H), 1.53-1.43 (m, 1H), 1.29 (m, 1H), 0.91-0.78 (m, 6H), 0.65 (m, 2H), 0.61-0.53 (m, 2H).

Example 2: Synthesis of N-((S)-1-(((S)-4-(cyclopropylamino)-3,4-dioxo-1-phenylbutan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-3-methoxybenzamide



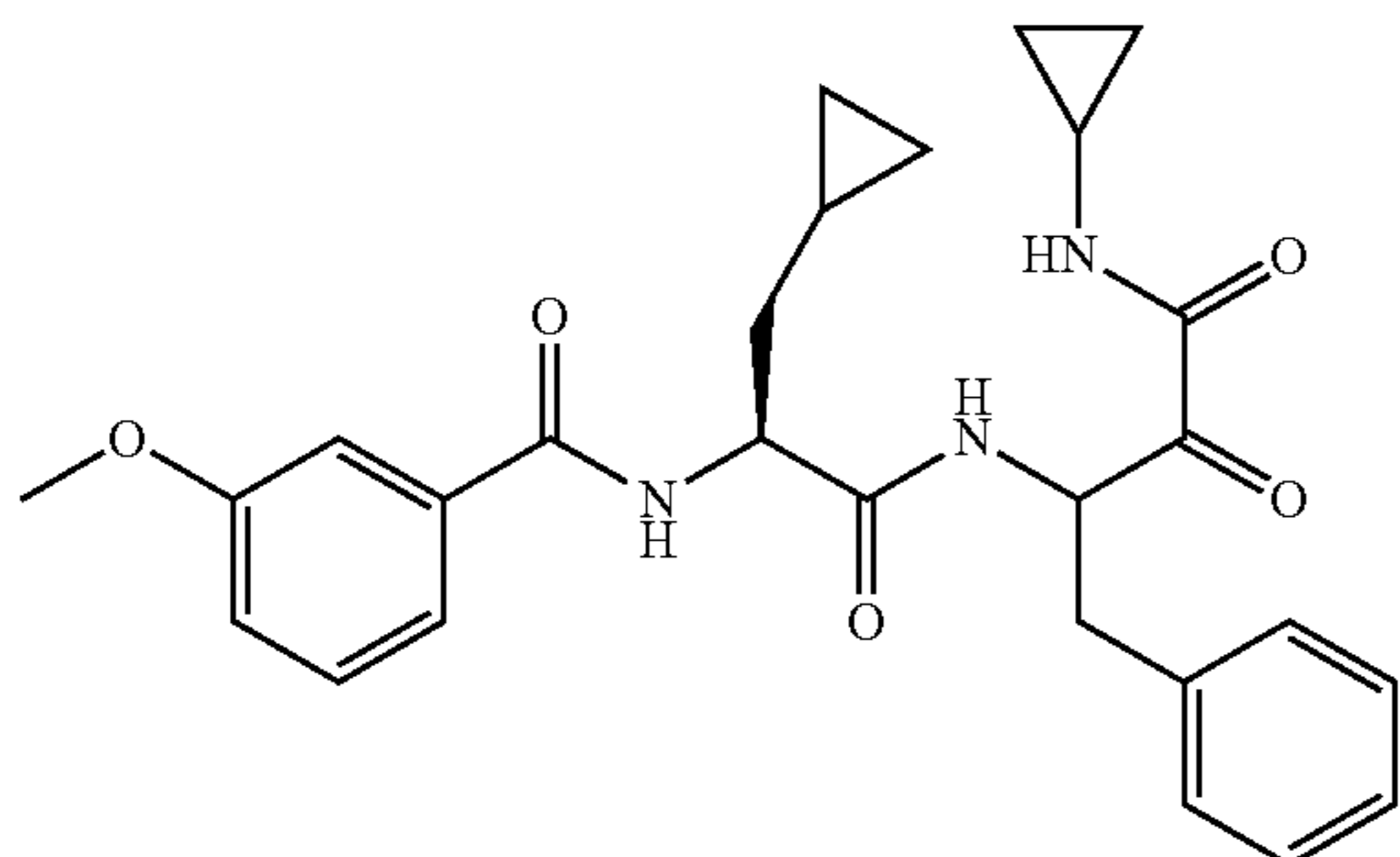
Dess-Martin Periodinane 15% solution in DCM (3.13 ml, 1.51 mmol, 1.5 eq) was added to a solution of intermediate 10 (470 mg, 1 mmol, 1.0 eq) dissolved in a previously prepared solution in DCM (10 ml) and of water (5 μ l). The mixture was stirred for 15 minutes at room temperature. After this time, 1.5 eq of Dess-Martin Periodinane 15% solution were added and mixture stirred for a further 15 minutes at room temperature. The volatiles were removed under vacuum and the crude mixture was extracted with EtOAc and washed with a 1 M solution of NaOH. The crude product was triturated using hexane and filtered. 352 mg of product was obtained (0.76 mmol, 76% yield).

LC-MS: $t_R=2.52$ min; $m/z=466$

$^1\text{H NMR}$ (400 MHz, DMSO-d_6) δ 0.61 (s, 2H), 0.61-0.68 (m, 2H), 0.73 (dd, $J=23.0$, 6.7 Hz, 3H), 0.87 (dd, $J=6.7$, 1.8 Hz, 3H), 1.92-2.10 (m, 1H), 2.68-2.85 (m, 2H), 3.07-3.18 (m, 1H), 3.81 (d, $J=1.1$ Hz, 3H), 4.32 (td, $J=8.3$, 5.6 Hz, 1H), 5.18-5.28 (m, 1H), 7.10 (dddd, $J=8.0$, 2.8, 1.9, 1.1 Hz, 1H), 7.15-7.25 (m, 5H), 7.34-7.46 (m, 3H), 8.15 (dd, $J=11.8$, 9.0 Hz, 1H), 8.45 (dd, $J=14.4$, 7.2 Hz, 1H), 8.76 (dd, $J=21.8$, 5.1 Hz, 1H).

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Example 3: Synthesis of N-((S)-3-cyclopropyl-1-((4-(cyclopropylamino)-3,4-dioxo-1-phenylbutan-2-yl)amino)-1-oxopropan-2-yl)-3-methoxybenzamide

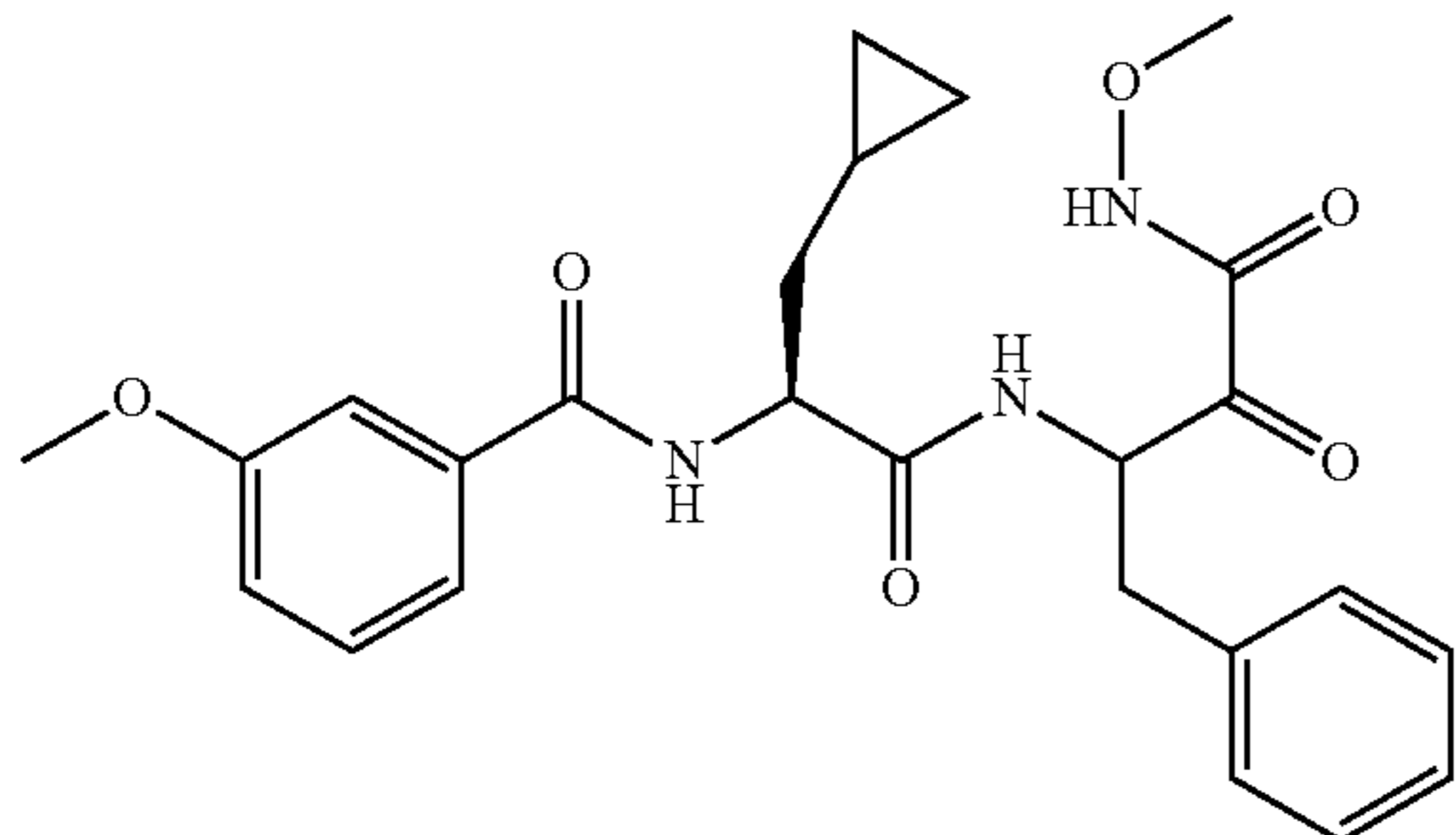


Dess-Martin Periodinane 15% solution in DCM (2.73 ml, 1.31 mmol, 1.5 eq) was added to a solution of Intermediate 13 (420 mg, 0.88 mmol, 1.0 eq) dissolved in 8 ml of a previously prepared solution based in DCM (10 ml) and 5 μ l of water. The mixture was stirred for 15 minutes at room temperature. After this time, 1.5 eq of Dess-Martin Periodinane 15% solution were added and mixture stirred for a further 15 minutes at room temperature. The volatiles were removed under vacuum and the crude mixture was extracted with EtOAc and washed with a 1 M solution of NaOH. The crude product was triturated using hexane and filtered. 274 mg of product was obtained (0.57 mmol, 65% yield).

LC-MS: $t_R=2.57$ min; $m/z=478$

$^1\text{H NMR}$ (400 MHz, DMSO- d_6) δ -0.01-0.20 (m, 2H), 0.25-0.43 (m, 1H), 0.54-0.70 (m, 4H), 0.70-0.79 (m, 1H), 1.18-1.35 (m, 1H), 1.47-1.68 (m, 2H), 2.69-2.88 (m, 2H), 3.12 (td, $J=13.5, 4.2$ Hz, 1H), 3.80 (d, $J=1.2$ Hz, 3H), 4.55 (td, $J=8.7, 5.1$ Hz, 1H), 5.20 (dtd, $J=9.0, 7.8, 4.1$ Hz, 1H), 7.08-7.12 (m, 1H), 7.16-7.25 (m, 5H), 7.34-7.46 (m, 3H), 8.29-8.45 (m, 2H), 8.75 (dd, $J=27.7, 5.1$ Hz, 1H).

Example 4: Synthesis of N-((S)-3-cyclopropyl-1-((4-(methoxyamino)-3,4-dioxo-1-phenylbutan-2-yl)amino)-1-oxopropan-2-yl)-3-methoxybenzamide



Dess-Martin Periodinane 15% solution in DCM (0.827 ml, 0.4 mmol, 1.5 eq) was added in 4 portions to a solution of intermediate 21 (170 mg, 0.36 mmol, 1.0 eq) in DMSO (2.5 ml) stirring the mixture for 10 minutes between additions. The mixture was stirred for 2 hours at room temperature and then the volatiles were removed under vacuum. The crude mixture was extracted with EtOAc and washed with a 1 M solution of NaOH. The volatiles were evaporated and the crude mixture was purified on reverse phase chroma-

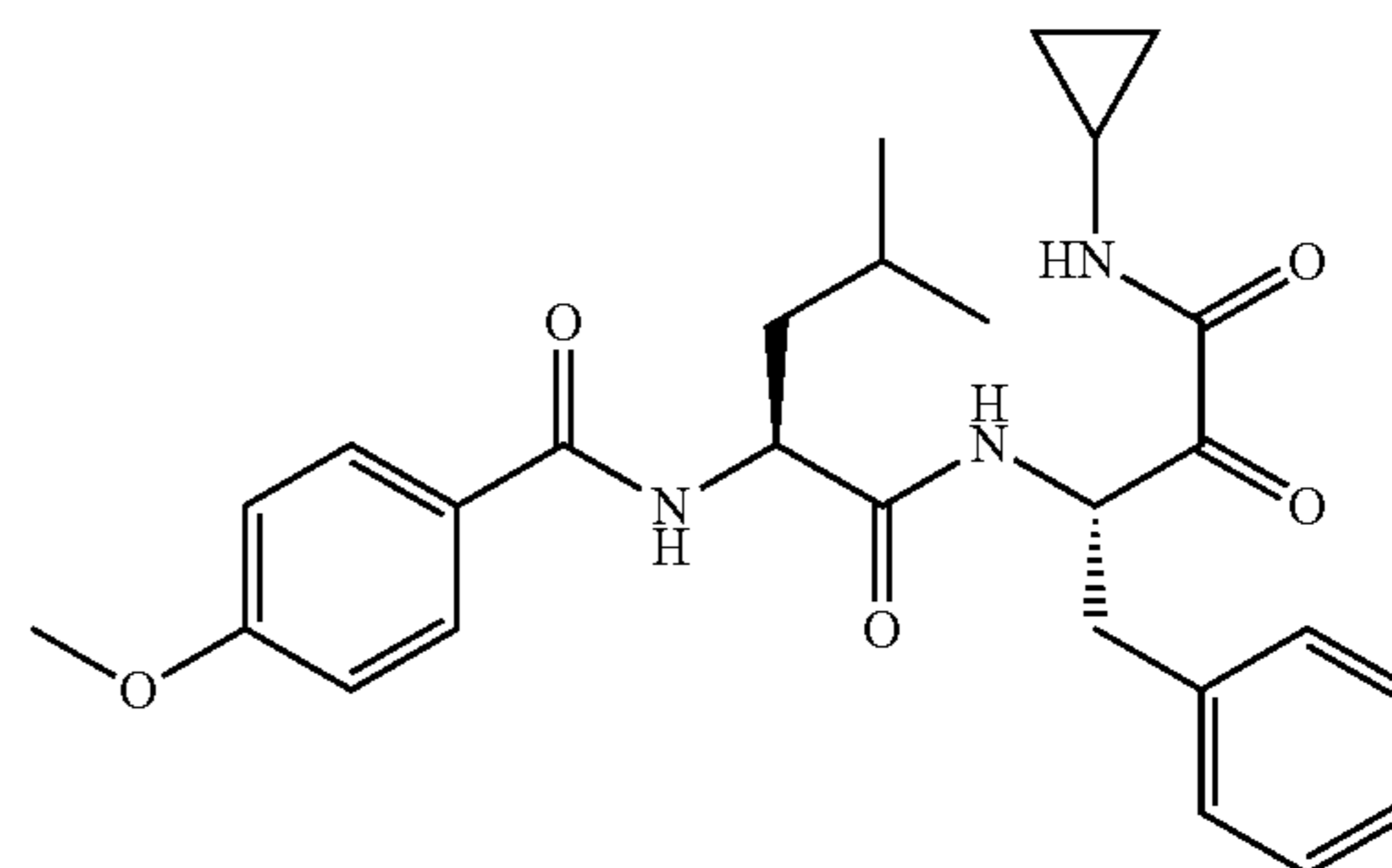
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tography (Biotage) using $\text{H}_2\text{O}/\text{Acetonitrile}$ as solvents, from 0% to 100% in Acetonitrile, product eluted at 20%. 14.2 mg of product were obtained (0.03 mmol, 8% yield).

LC-MS: $t_R=2.03$ min; $m/z=468$

$^1\text{H NMR}$ (400 MHz, DMSO- d_6) δ -0.04-0.23 (m, 1H), 0.34 (ddd, $J=17.7, 9.1, 5.5$ Hz, 1H), 1.43-1.68 (m, 1H), 2.52 (d, $J=1.9$ Hz, 1H), 2.67-2.83 (m, 1H), 3.10-3.22 (m, 2H), 3.65 (d, $J=13.1$ Hz, 2H), 3.79 (d, $J=2.2$ Hz, 3H), 4.53 (tt, $J=9.0, 4.6$ Hz, 1H), 5.32 (s, 1H), 6.95-7.33 (m, 6H), 7.33-7.46 (m, 3H), 8.09 (d, $J=72.5$ Hz, 1H), 8.34 (dd, $J=26.3, 8.3$ Hz, 1H).

Comparative Example 5: N-((S)-1-(((S)-4-(cyclopropylamino)-3,4-dioxo-1-phenylbutan-2-yl)amino)-4-methyl-1-oxopentan-2-yl)-4-methoxybenzamide

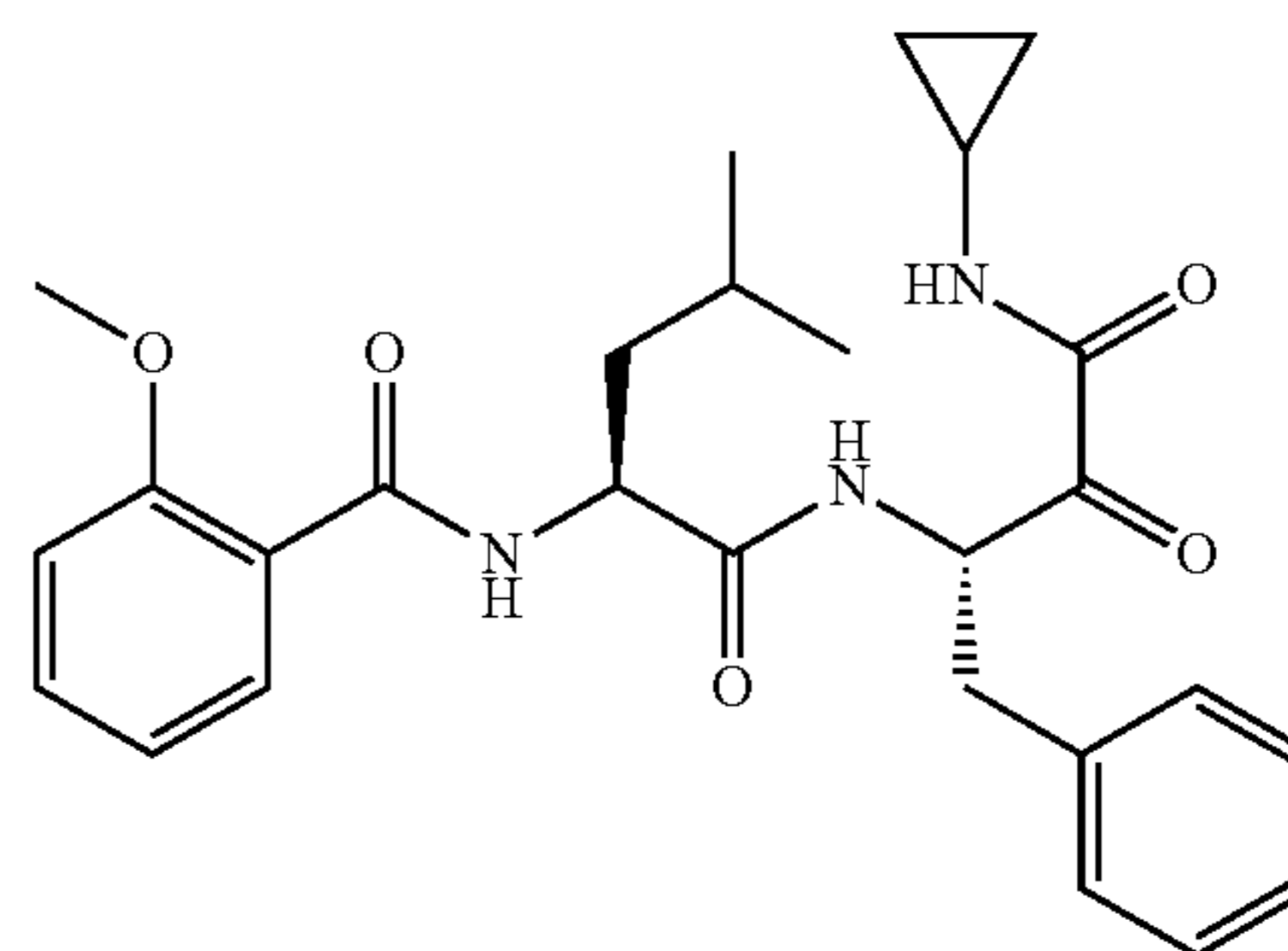


5 μ L of water were added to Dess-Martin Periodinane 15% solution in DCM (744 μ l, 0.3 mmol, 1.5 eq). Then the mixture was added to a solution of intermediate 22 (115 mg, 0.2 mmol, 1.0 eq.) in DCM (5 ml). The mixture was stirred for 15 minutes at room temperature. Volatiles were removed under vacuum. Crude was extracted with EtOAc and washed with a sat. solution of NaHCO_3 (2 \times 10 mL). Crude was purified on flash chromatography (ISCO Rf) using Hexane/Hexane:Ethanol (8:2) as solvents, from 0% to 30% in Hexane:Ethanol (8:2), product eluted at 20%. 31.2 mg were obtained (0.2 mmol, 27%).

LC-MS: $t_R=2.60$ min; $m/z=480$

$^1\text{H NMR}$ (400 MHz, DMSO- d_6) δ 8.74 (dd, $J=25.5, 5.1$ Hz, 1H), 8.33 (dd, $J=34.8, 7.4$ Hz, 1H), 8.19 (dd, $J=14.2, 8.4$ Hz, 1H), 7.88-7.82 (m, 2H), 7.24-7.14 (m, 5H), 7.03-6.95 (m, 2H), 5.17 (m, 1H), 4.52 (m, 1H), 3.81 (s, 3H), 3.12 (m, 1H), 2.91-2.74 (m, 1H), 2.74-2.65 (m, 1H), 1.60 (m, 1H), 1.48 (m, 1H), 1.30 (m, 1H), 0.92-0.78 (m, 6H), 0.68-0.61 (m, 2H), 0.58 (m, 2H).

Comparative Example 6: N-((S)-1-(((S)-4-(cyclopropylamino)-3,4-dioxo-1-phenylbutan-2-yl)amino)-4-methyl-1-oxopentan-2-yl)-2-methoxybenzamide



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5 μ L of water were added to a Dess-Martin Periodinane 15% solution in DCM (1.6 ml, 0.7 mmol, 1.5 eq). Then the mixture was added to a solution of intermediate 23 (250 mg, 0.5 mmol, 1.0 eq.) in DCM (10 ml). The mixture was stirred for 15 minutes at room temperature. The volatiles were removed under vacuum and the crude mixture was extracted with EtOAc and washed with a sat. solution of NaHCO₃ (2x10 mL). The crude product was purified on flash chromatography (ISCO Rf) using Hexane/Hexane:Ethanol (8:2) as solvents, from 0% to 30% in Hexane:Ethanol (8:2), product eluted at 20%. 89.2 mg of product was obtained (0.2 mmol, 35%).

LC-MS: t_R =2.68 min; m/z =480

¹H NMR (400 MHz, DMSO-d₆) δ 8.78 (dd, J =19.7, 5.1 Hz, 1H), 8.51 (dd, J =32.3, 7.5 Hz, 1H), 8.26 (dd, J =8.2, 6.1 Hz, 1H), 7.79 (m, 1H), 7.49 (m, 1H), 7.25-7.21 (m, 4H), 7.20-7.13 (m, 2H), 7.05 (m, 1H), 5.24-5.17 (m, 1H), 4.63-4.55 (m, 1H), 3.86 (s, 3H), 3.15 (m, 1H), 2.85-2.75 (m, 1H), 2.75-2.70 (m, 1H), 1.66-1.46 (m, 2H), 1.36 (m, 1H), 0.91-0.77 (m, 6H), 0.67-0.62 (m, 2H), 0.61-0.55 (m, 2H).

Biological Data

Assay for the Determination of the Capacity to Inhibit Calpain 1

Activity of Calpain 1 was measured by using Calpain-Glo Protease kit from Promega. Assays were run in 384-well black plates with clear bottom. 5 μ L of compounds in a 10 mM HEPES and 1 mM EDTA aqueous solution were incubated with 15 μ L of a solution of 16 ng/mL calpain 1 (Sigma Aldrich) for 2 minutes and then 20 μ L of Calpain-Glo™ Reagent provided in the kit were added to each well. Luminescence elicited by calpain 1-induced substrate lysis was measured in a Hamamatsu FDSS 7000 reader and data was calculated from the maximal luminescence peak. Calpain inhibition was calculated from the formula:

$$\% \text{ inhibition} = (LT - LB) * 100 / (LC - LB);$$

wherein LT is the luminescence observed in the tested compound, LB is blank luminescence obtained from a well without calpain 1 and LC is the luminescence observed in control wells including calpain 1 in the absence of inhibitor.

All compounds were tested using, at least, 6 concentrations to allow calculate the IC₅₀ (concentration that inhibits the activity of calpain 1 by 50%).

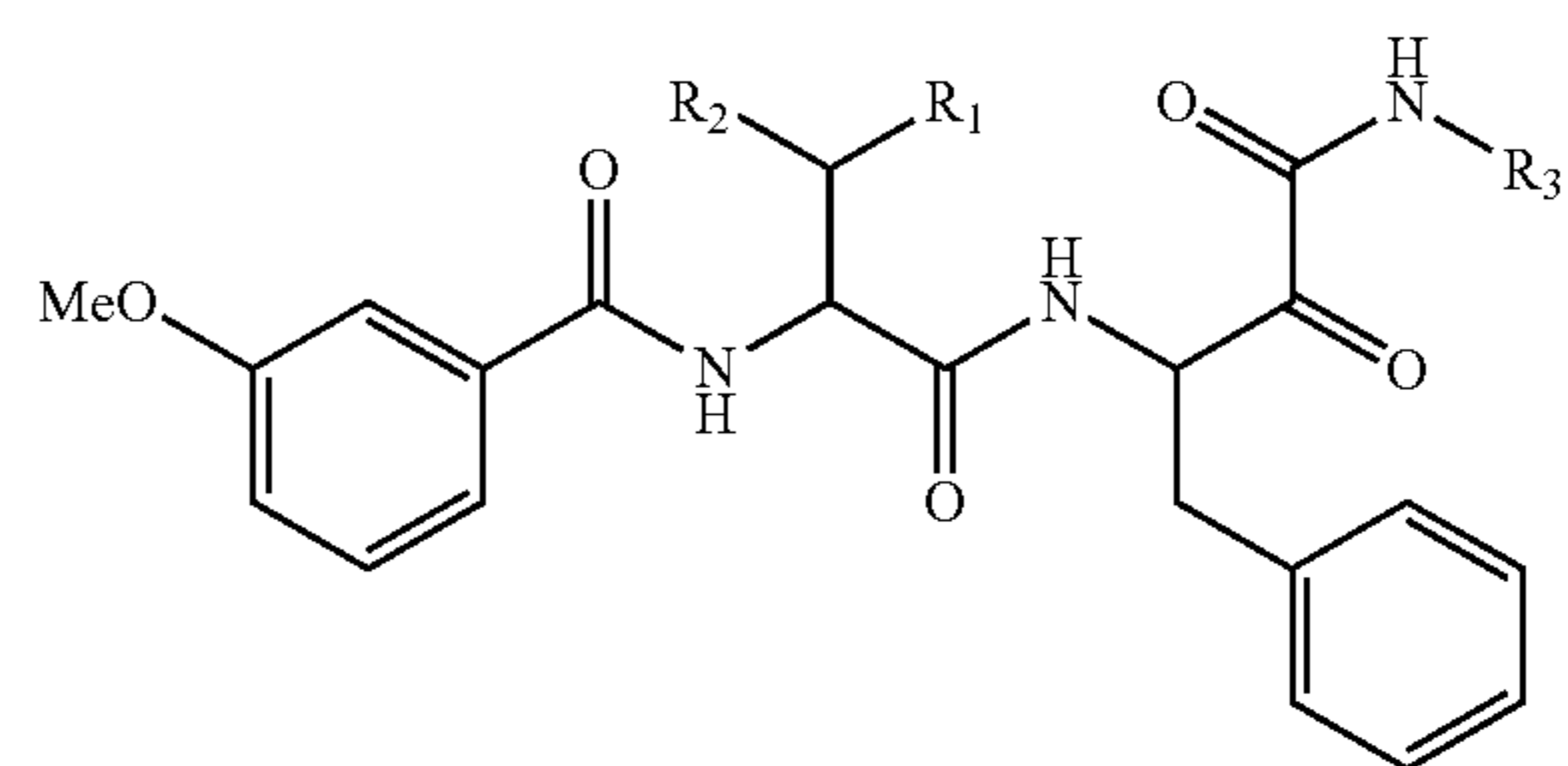
Calpastatin and ALLN were included as control inhibitors in all the assays.

Compound	Calpain 1 inhibitory activity IC ₅₀ (nM)
Example 1	0.03
Example 2	2.2
Example 3	0.008
Example 4	7.1
Comp. Example 5	255
Comp. Example 6	110

The invention claimed is:

1. A method for the treatment of a disease or condition associated with elevated calpain 1 activity selected from the group consisting of heart injury caused by infarction; ischemia with or without reperfusion; neurodegenerative disorders; malaria; diabetic nephropathy; neurotoxicity induced by HIV virus; cancer; and fibrotic diseases, the method comprising administering to a subject in need thereof a therapeutically effective amount of a calpain 1 inhibitor of formula (I):

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wherein

R₁ is selected from the group consisting of C₁-C₆ alkyl and C₃-C₆ cycloalkyl,

R₂ is selected from the group consisting of H and C₁-C₆ alkyl, and

R₃ is selected from the group consisting of C₁-C₆ alkoxy and C₃-C₆ cycloalkyl,

or a pharmaceutically acceptable salt or stereoisomer thereof

or a pharmaceutical composition comprising said compound and a pharmaceutically acceptable excipient.

2. The method according to claim 1, wherein

R₁ is selected from the group consisting of C₁-C₃ alkyl and C₃-C₅ cycloalkyl,

R₂ is selected from the group consisting of H and C₁-C₃ alkyl, and

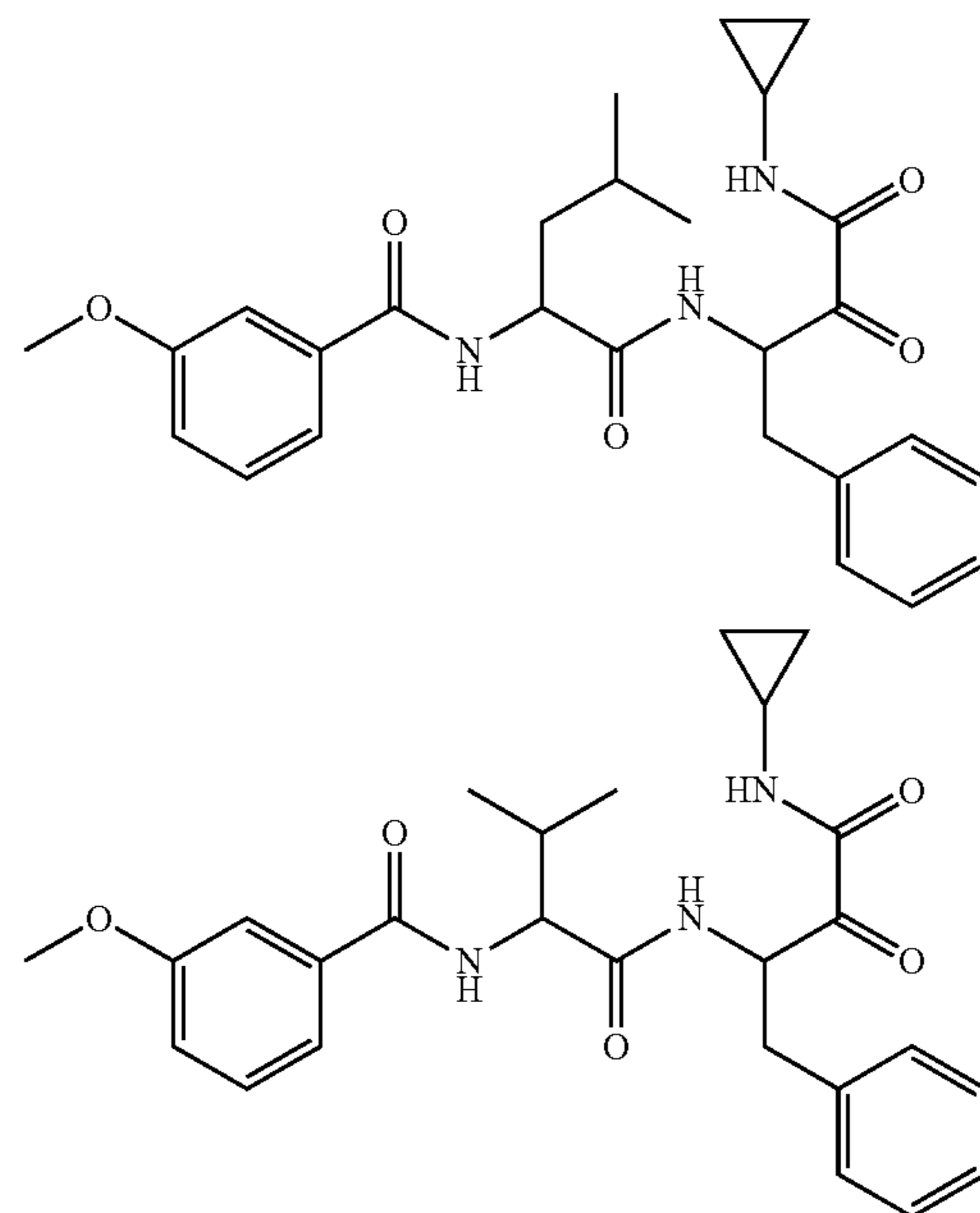
R₃ is selected from the group consisting of C₁-C₃ alkoxy and C₃-C₅ cycloalkyl.

3. The method according to claim 1, wherein R₁ is selected from the group consisting of methyl, isopropyl and cyclopropyl.

4. The method according to claim 1, wherein R₂ is selected from the group consisting of H and methyl.

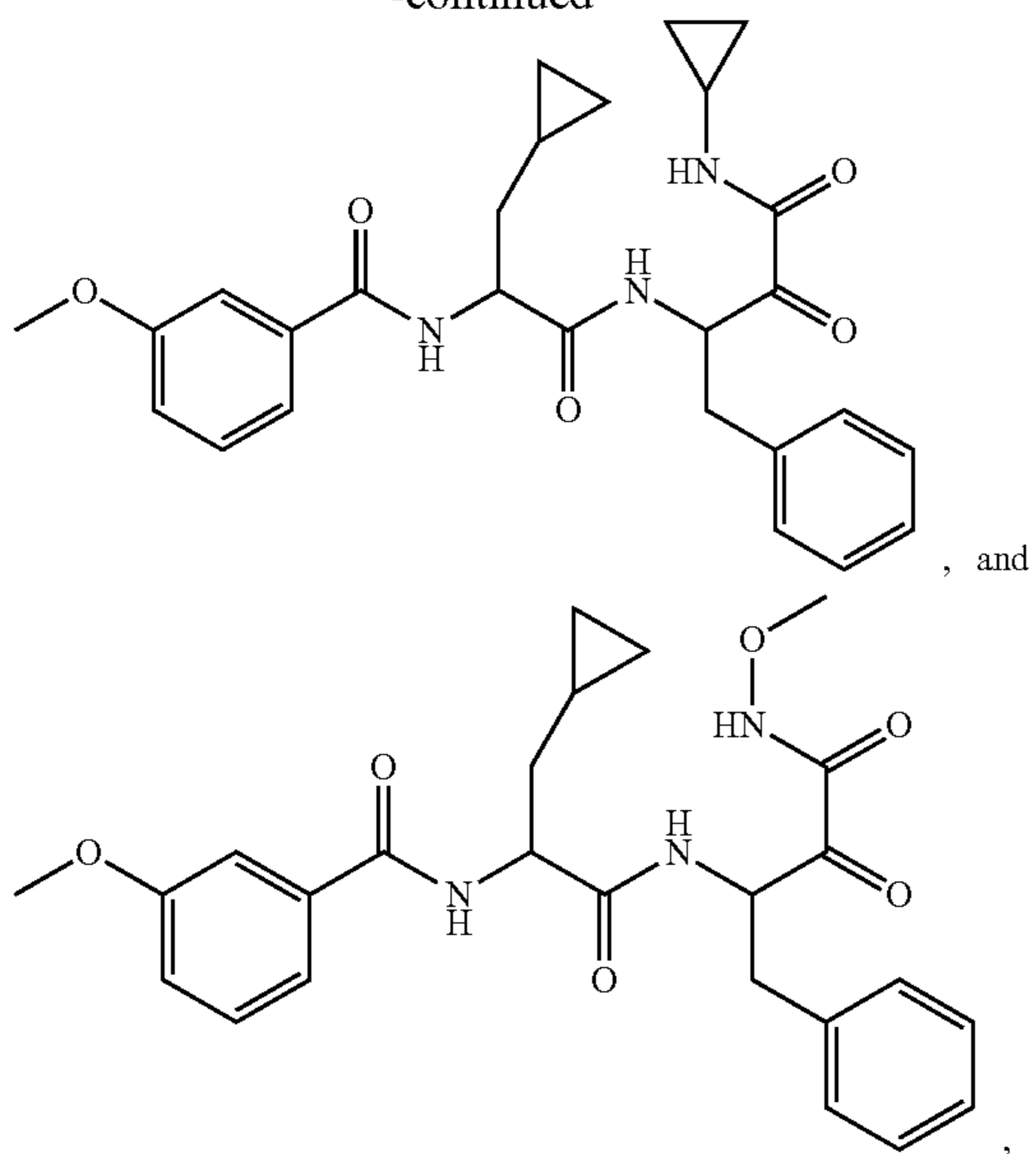
5. The method according to claim 1, wherein R₃ is selected from the group consisting of cyclopropyl and methoxy.

6. The method according to claim 1, wherein the compound of formula (I) is selected from the group consisting of:



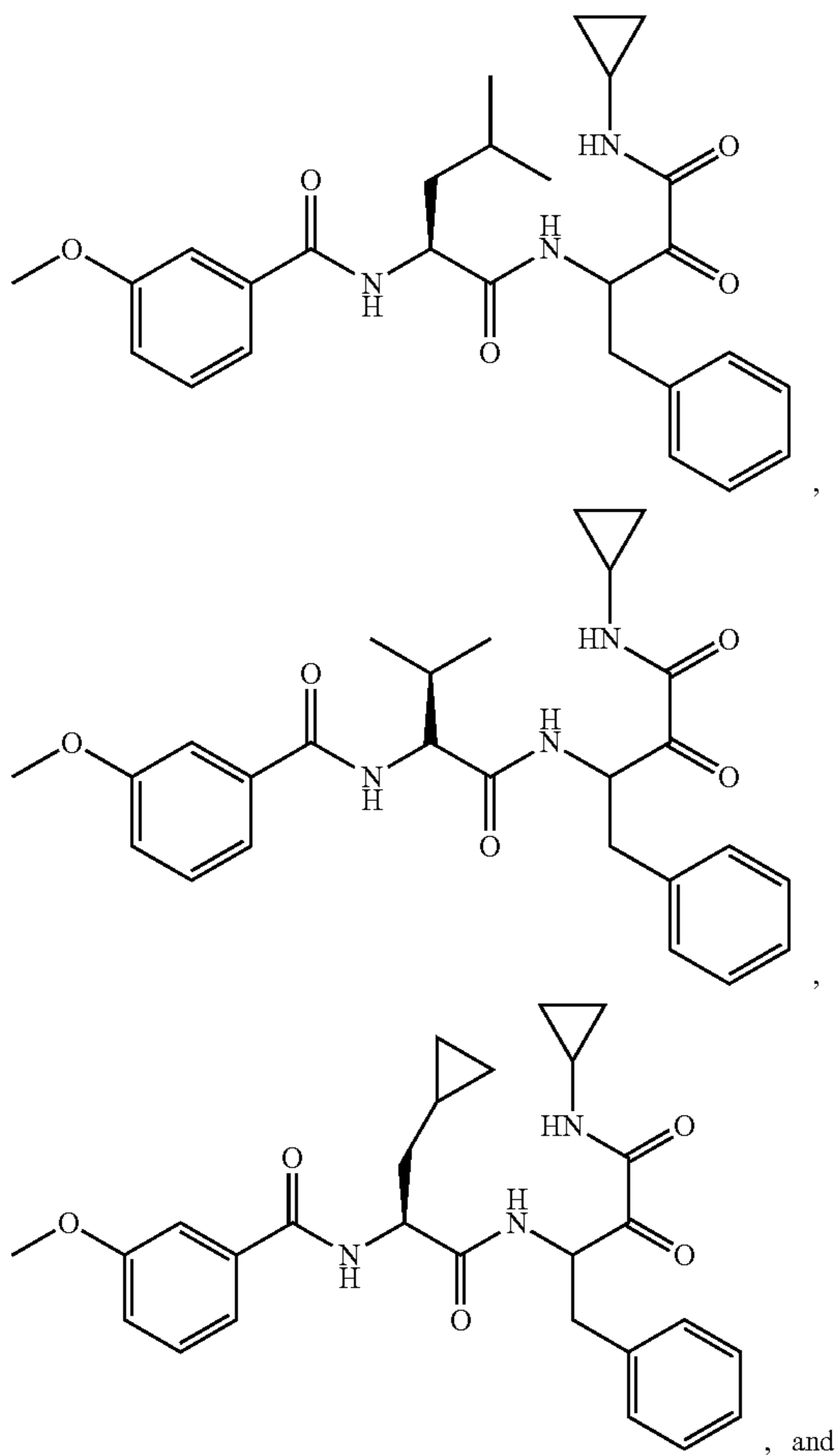
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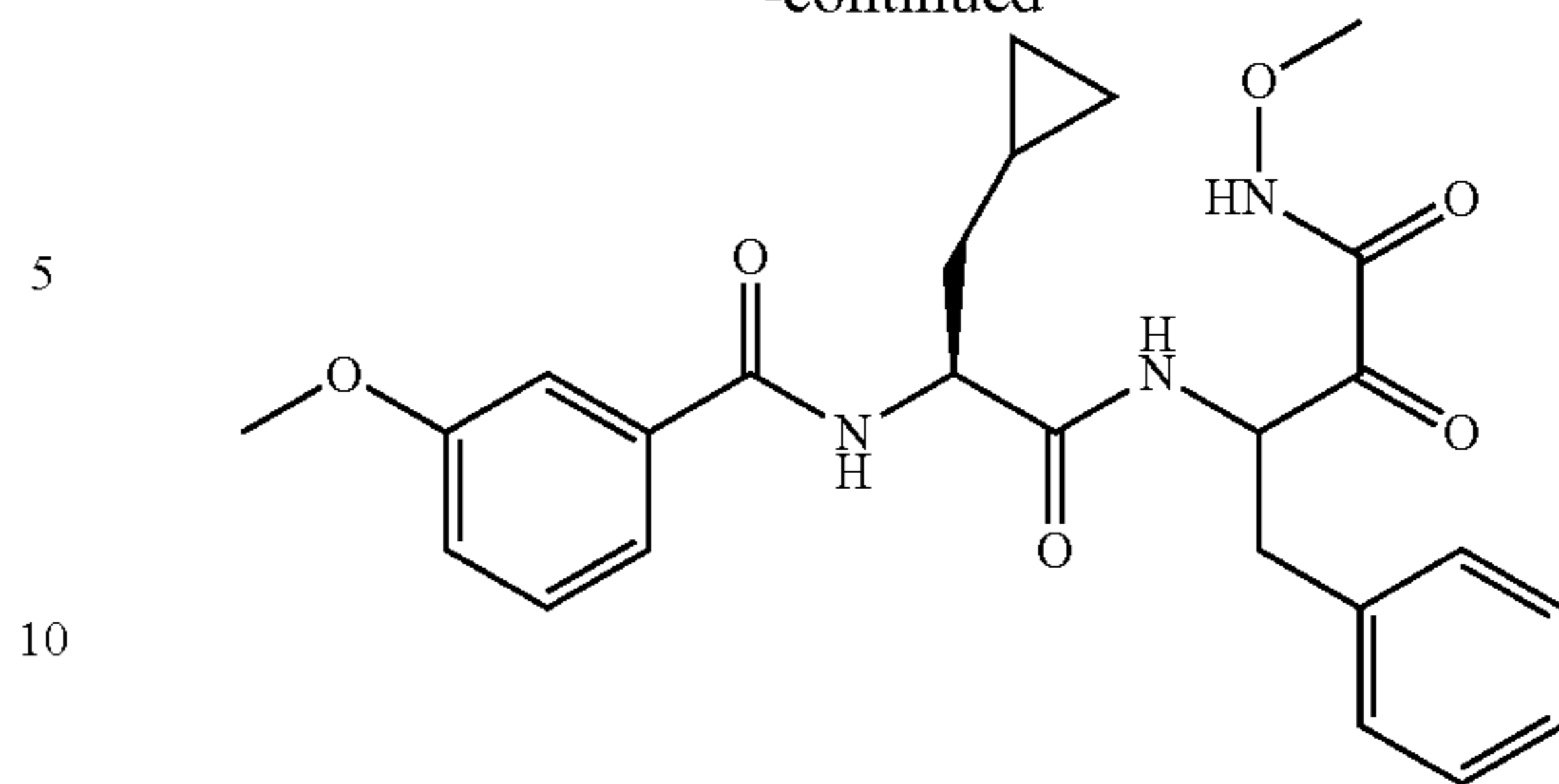
or a stereoisomer or pharmaceutically acceptable salt thereof.

7. The method according to claim 1, wherein the compound of formula (I) is selected from the group consisting of:



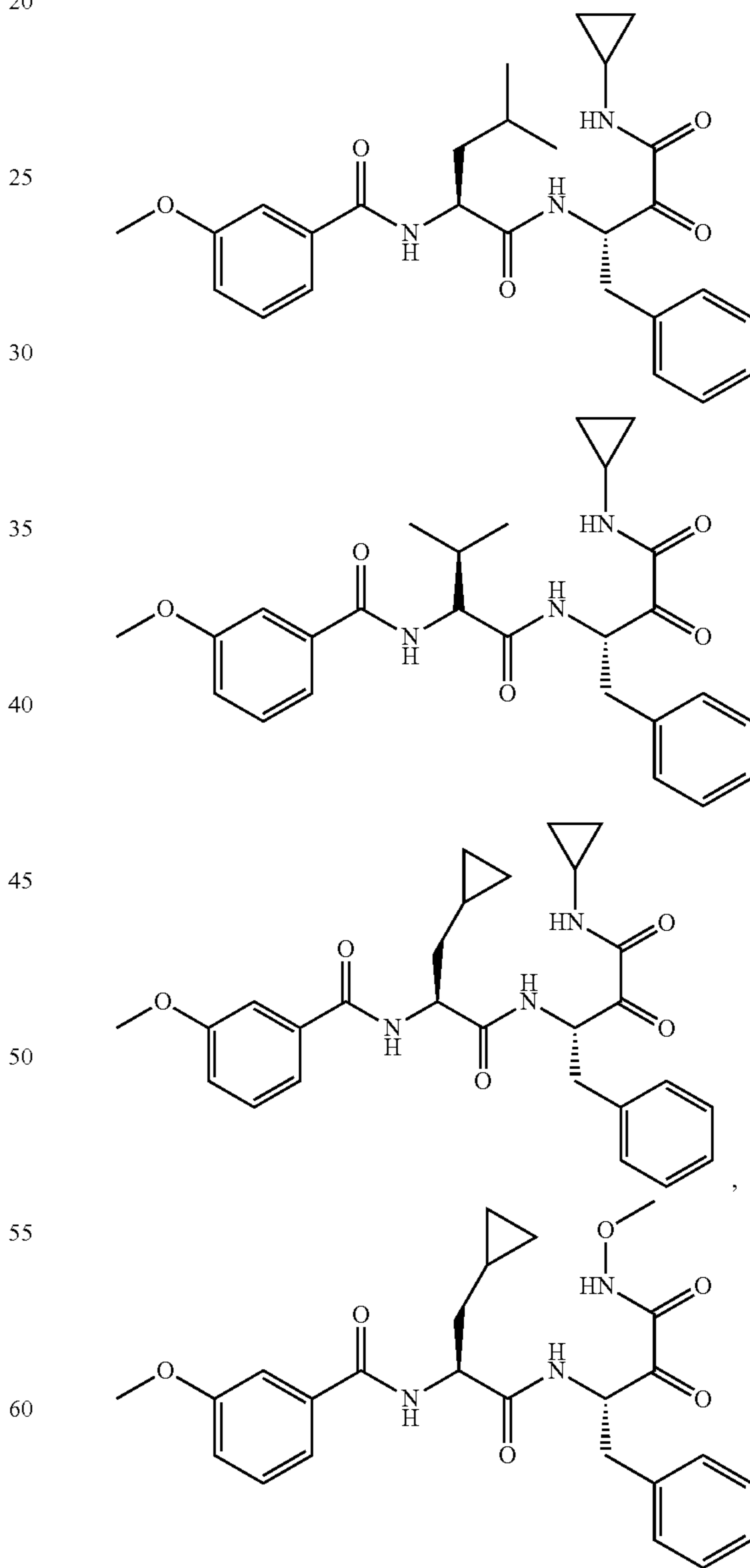
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or a stereoisomer or pharmaceutically acceptable salt thereof.

8. The method according to claim 1, wherein the compound of formula (I) is selected from the group consisting of:



or a pharmaceutically acceptable salt thereof.

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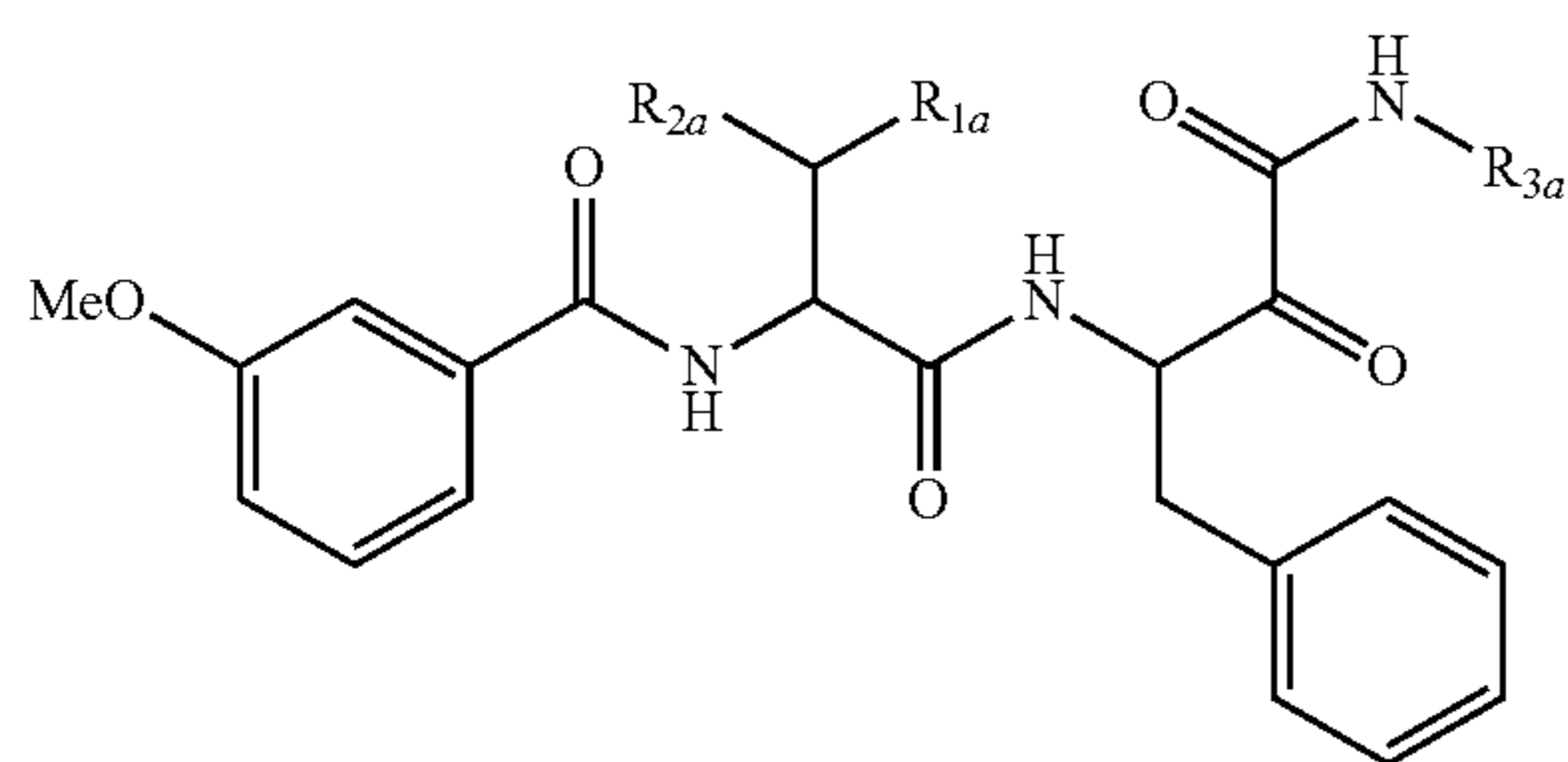
9. The method according to claim 1, wherein the disease or condition is heart injury caused by infarction or ischemia with or without reperfusion.

10. The method according to claim 9, wherein the heart injury is remodeling after myocardial infarction.

11. The method according to claim 1, wherein the neurodegenerative disorder is selected from the group consisting of Alzheimer's disease, Parkinson disease, multiple sclerosis, acute autoimmune encephalitis, and Creutzfeldt-Jakob disease; and/or cancer is selected from the group consisting of breast cancer, colorectal cancer and leukaemia.

12. The method according to claim 1, wherein the fibrotic disease is selected from the group consisting of cardiac fibrosis, lung fibrosis, liver fibrosis, renal fibrosis and retroperitoneal fibrosis.

13. A compound of formula (II):



wherein

R_{1a} is selected from the group consisting of C_1 - C_6 alkyl and C_3 - C_6 cycloalkyl,

R_{2a} is selected from the group consisting of H and C_1 - C_6 alkyl, and

R_{3a} is C_3 - C_6 cycloalkyl,

or a pharmaceutically acceptable salt or stereoisomer thereof.

14. The compound of formula (II) according to claim 13, wherein

R_{1a} is selected from the group consisting of C_1 - C_3 alkyl and C_3 - C_5 cycloalkyl,

R_{2a} is selected from the group consisting of H and C_1 - C_3 alkyl,

R_{3a} is C_3 - C_5 cycloalkyl.

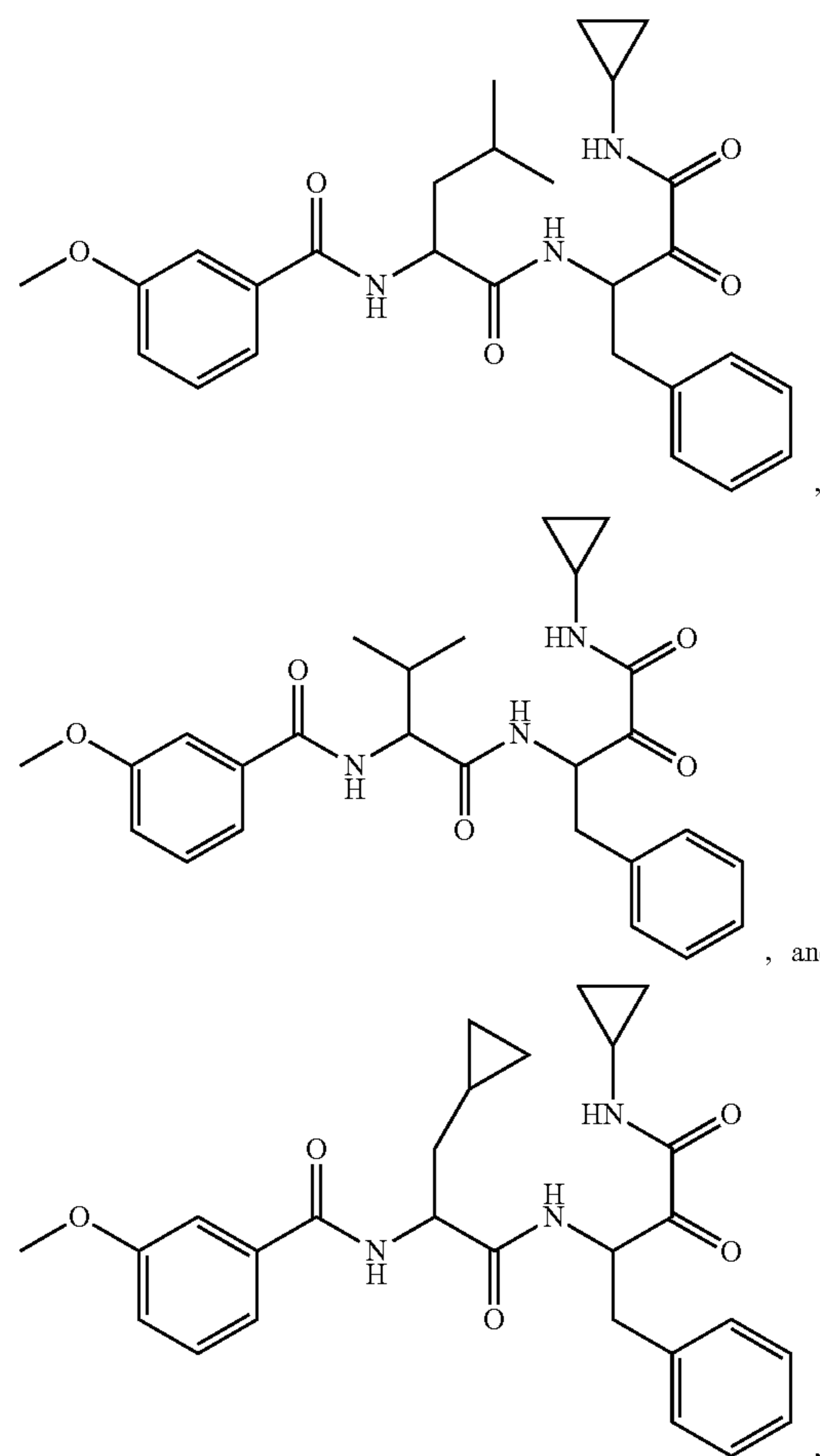
15. The compound of formula (II) according to claim 13, wherein R_{1a} is selected from the group consisting of methyl, isopropyl and cyclopropyl.

16. The compound of formula (II) according to claim 13, wherein R_{2a} is selected from the group consisting of H and methyl.

17. The compound of formula (II) according to claim 13, wherein R_{3a} is cyclopropyl.

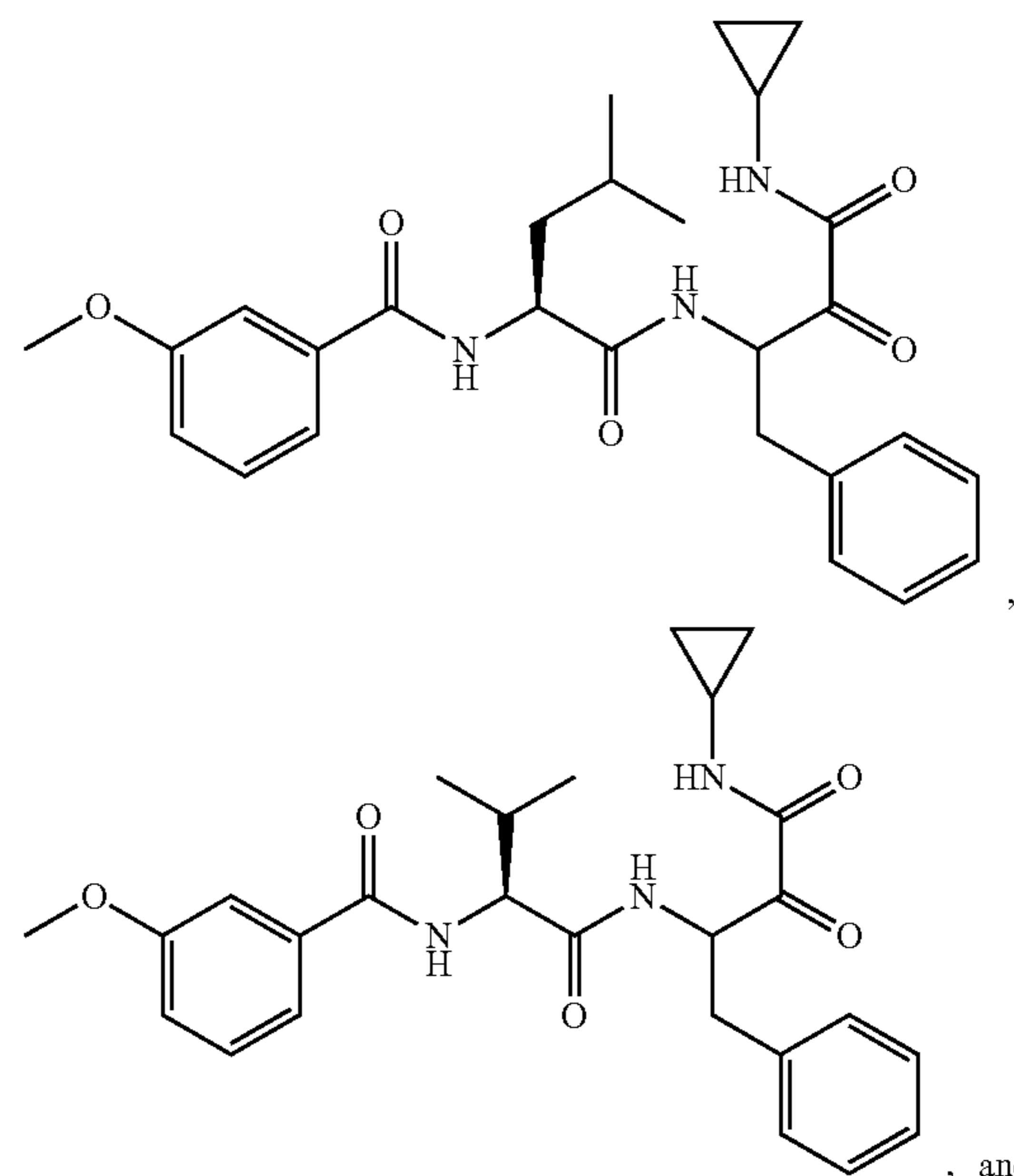
18. The compound of formula (II) according to claim 13, which is selected from the group consisting of:

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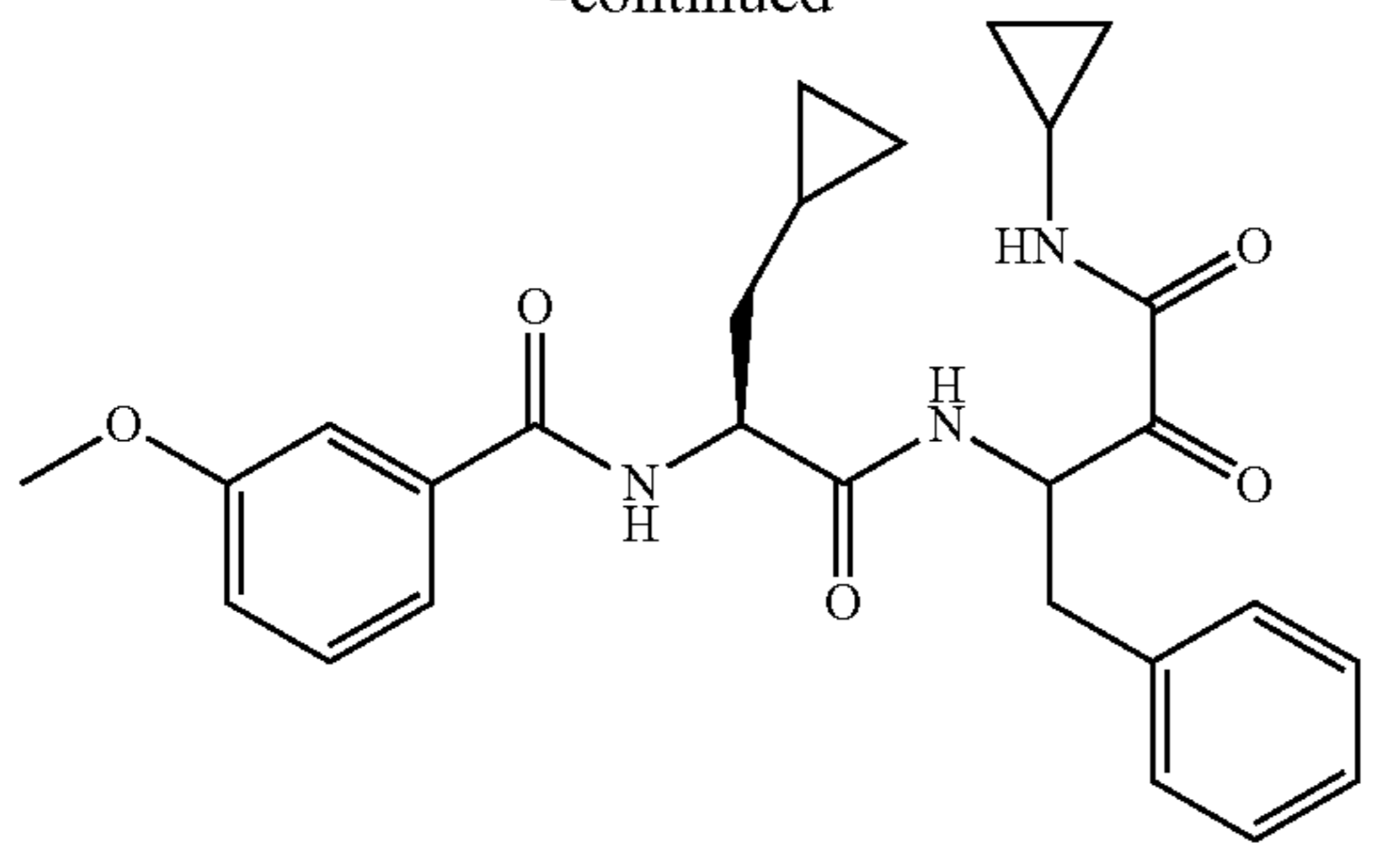
or a stereoisomer or pharmaceutically acceptable salt thereof.

19. The compound of formula (II) according to claim 13, which is selected from the group consisting of:



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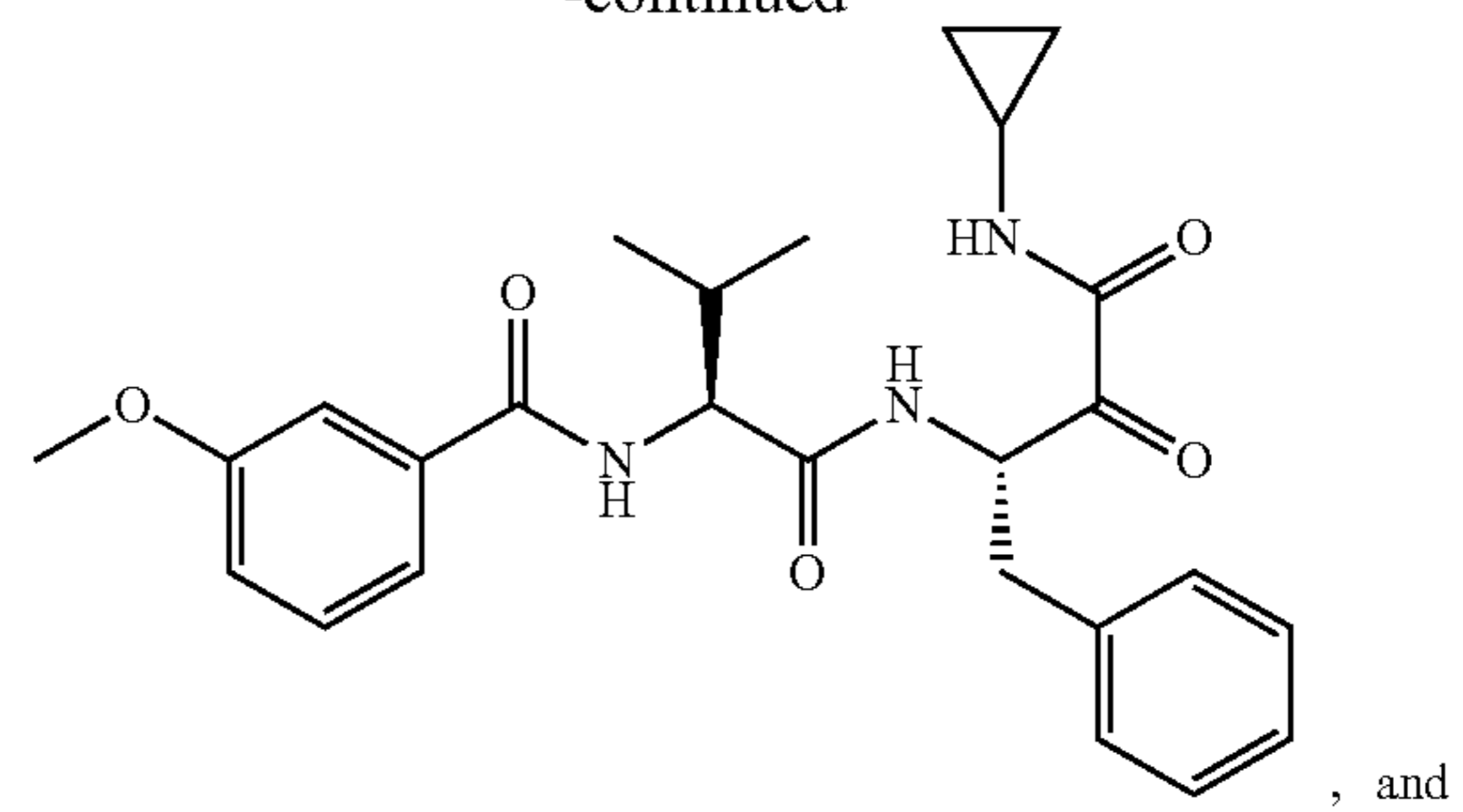


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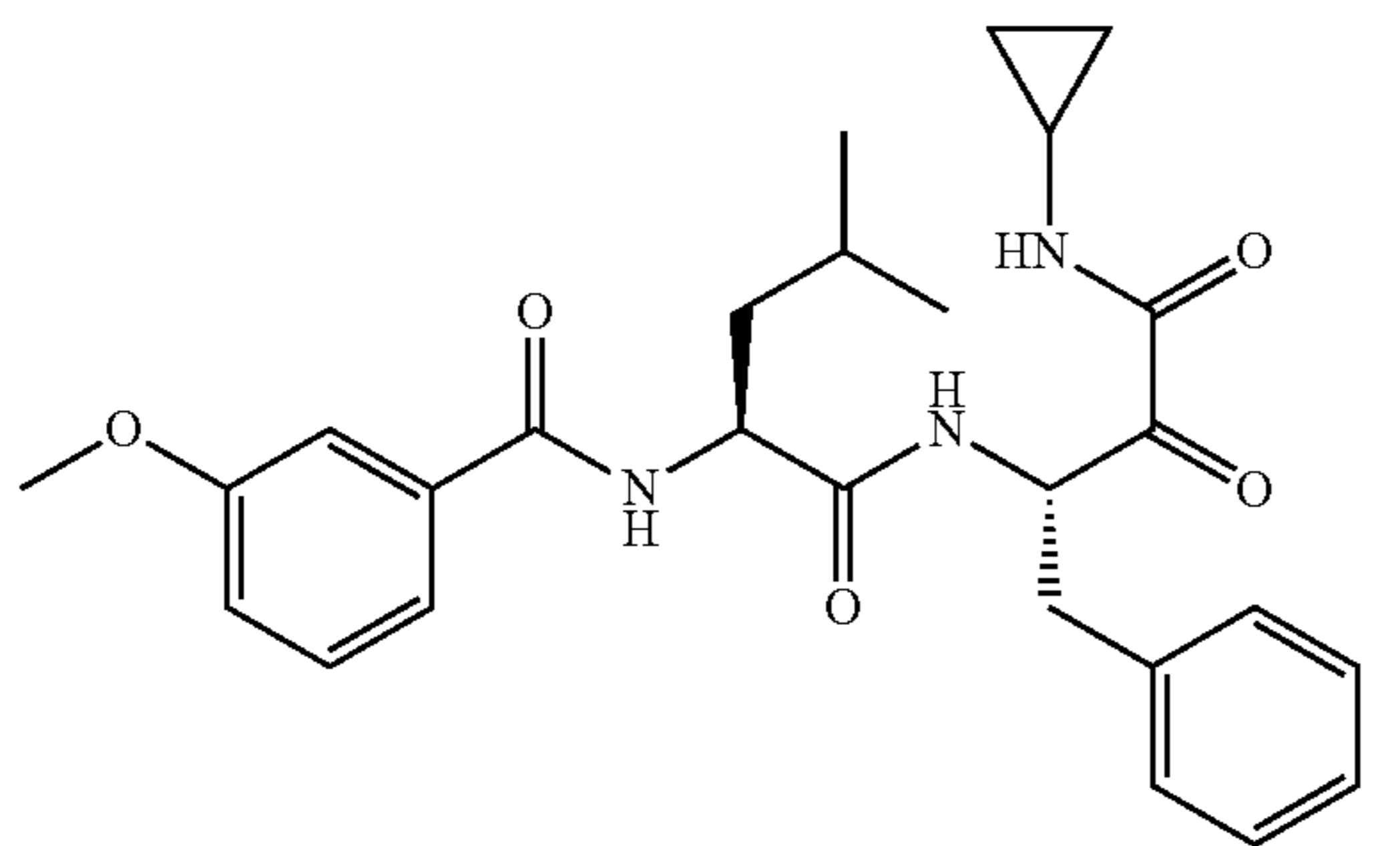
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, and

or a stereoisomer or pharmaceutically acceptable salt thereof.

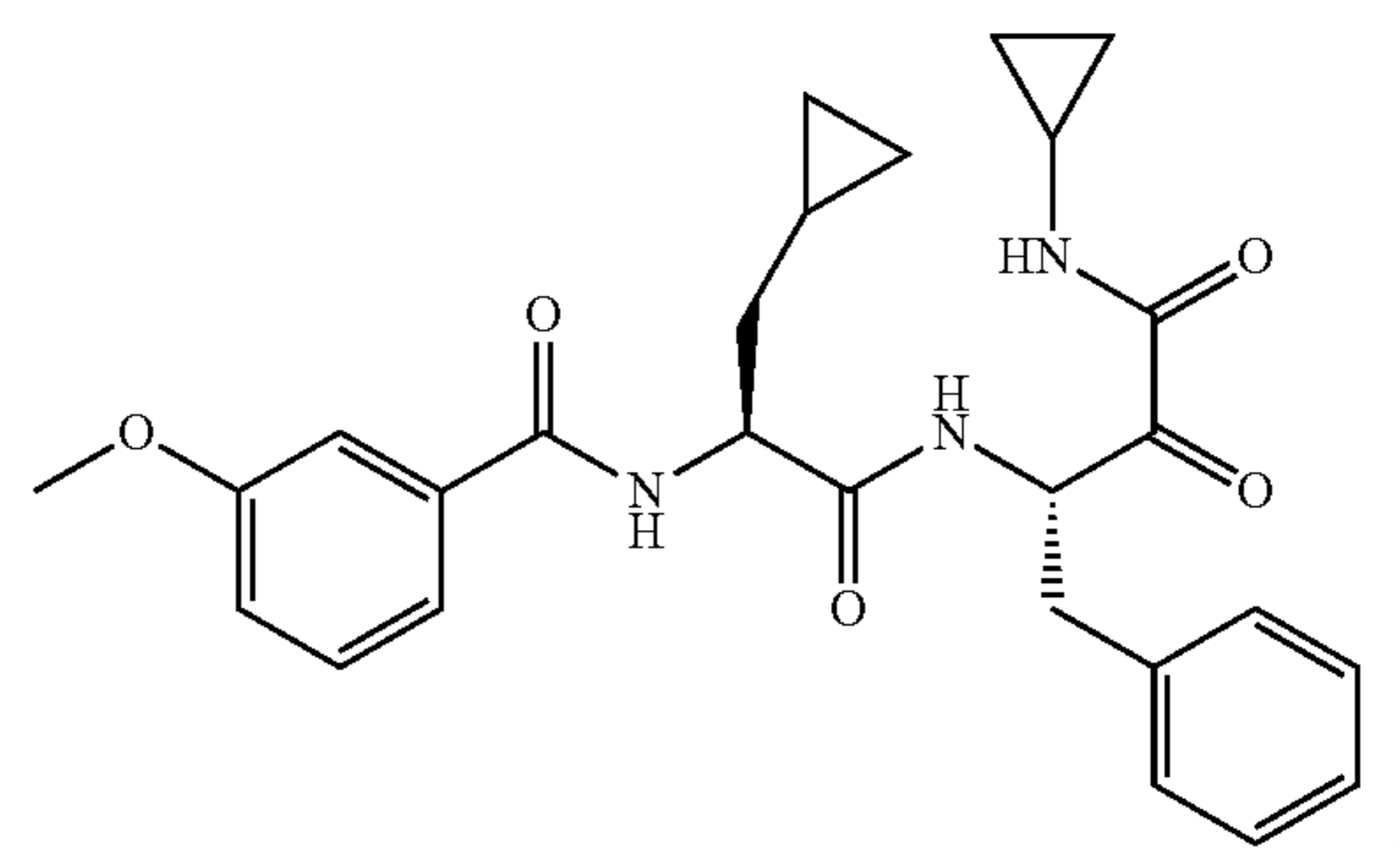
20. The compound of formula (II) according to claim **13**, which is selected from the group consisting of:



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or a pharmaceutically acceptable salt thereof.

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